The impact of two-socket preservation approaches on the soft and hard tissue healing: a short-term study in dogs

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Abstract

The present study was designed in the context of the uncertain circumstances related to the best therapeutic option for ridge preservation. The research aimed to investigate the quality of early healing processes developed in the former sockets preserved with a collagen matrix alone or associated with a bone substitute in comparison with naturally-healed sockets, using an animal model previously validated. In both quadrants of the mandible of two dogs, the distal sockets of the second and fourth premolars served as experimental sites. Two sockets healed naturally, three sockets were preserved with the collagen matrix and three sockets were preserved with the collagen matrix plus a bone substitute. After one month of healing, the samples were harvested and histologically processed. The soft tissue covering the preserved ridges displayed an obviously thicker epithelial layer containing mostly areas of parakeratinized epithelium alternating with keratinized ones in comparison with naturally-healed sockets. In the apical third of the sockets, a mature bone structure was recorded for all three types of post-extraction approaches. While in naturally-healed and collagen matrix-preserved specimens the central third of the sockets contained bone with a mature aspect, in collagen matrix plus bone substitute-preserved sockets an immature appearance was observed. In the external third of the sockets, only in matrix-preserved alveoli a well-developed cancellous bone was present. The bovine bone substitute seemed to delay hard tissue development. The use of the collagen matrix could be a clinical option to preserve post-extraction ridges especially when an improvement in soft tissue quality is desired.

Keywords: tooth extraction, bone substitute, collagen, barrier membrane.

Introduction

Alveolar bone resorption after tooth extraction induces changes in size and shape of the alveolar ridges. The height and width reduction of the alveolar ridge complicates the implant placement thereafter, in terms of aesthetics and functionality, especially in the anterior areas [1]. The healing of the extraction sockets is expected to decrease the alveolar ridge by 3.79±0.23 mm horizontally and 1 mm vertically [2]. Recent animal experiments describing the early healing events within the alveolar sockets after tooth extraction reported a marked horizontal reduction at the buccal bone wall in the most coronal aspect of the ridge accounting for about 40% of its initial dimension [3]. The implant placement immediately after tooth extraction could clinically maintain only part of the original bone volume [4]. There is strong evidence that the management of the sockets after tooth extraction using different biomaterials significantly reduces post-extraction resorption [2, 5, 6] and seems to be beneficial for further rehabilitations [7]. Therefore, in order to preserve the original ridge dimensions following tooth extraction as much as possible and promote qualitative bone regeneration of the former sockets, the use of various bone grafts and barrier membranes in socket preservation therapies has been suggested [8–10]. The use of membranes seems to be of particular interest as many studies have sustained their efficacy for preserving mostly the horizontal dimensions of the ridges [10].

Membranes used for guided bone regeneration should have several physical and biochemical properties in order to fulfill the barrier function, such as biocompatibility, tissue integration, exclusion of the gingival tissue from the regenerating site, space-making ability, shape adaptability and simple clinical maneuverability [11]. Non-resorbable membranes fulfill many of these requirements, but their main limitations are the need for additional surgery for their retrieval and the high rate of early exposure [12]. Collagen membranes have been largely used, but their fast resorption rate has raised concerns over their usage in guided bone regeneration procedures. Collagen cross-linking increases the biodurability of the material and enables collagen-based membranes to support long-term osteogenic activity while preventing other tissues from invading the defect [13]; however, a decreased biocompatibility, especially when cross-linking is mediated by glutaraldehyde, has been reported [14, 15]. Free gingival graft has been successfully used as an alternative to membranes for covering the extraction sockets, in humans, in order to assure primary wound closure and to minimize the buccal contour shrinkage [16]. The need of a second
operatory site and the increased possibility of graft necrosis orient the clinicians towards other options.

Recently, a new bilayer collagen matrix was designed and successfully used as an alternative to autologous soft tissues for the coverage of gingival recessions [10, 17] and for the increase of the width of keratinized gingiva [18, 19]. Because of its considerable thickness in comparison with conventional collagen membranes, the new collagen matrix might also provide supplementary protection of the post-extraction remodeling processes and possibly a supplementary space for soft tissue gain. The quality of the soft tissue covering the alveolar ridges is an important issue to be considered for further implant treatment and for the maintenance of healthy peri-implant tissues [20, 21]. One could remark the lack of studies recording the soft tissue behavior after different socket preservation approaches. In fact, a lot of recent research on this topic failed to include soft tissue dimensional changes as one of the outcomes of the study [22–25]. A few studies have reported good results on soft tissue healing when a single collagen membrane, or one in association with other biomaterials, was used in guided bone regeneration approaches [26, 27].

Therefore, the aim of the present animal study was to investigate the quality of early healing processes developed at the level of the soft and hard tissues of the former sockets preserved with a collagen matrix alone or in association with a bone substitute in comparison with naturally-healed sockets, using an animal model previously validated by our team [24, 27]. The collagen matrix was chosen as its thickness could induce a well-developed soft tissue and also enhance its barrier protecting properties eliminating the need to use double-layer bioresorbable membranes associated with increased operative time. The option to associate a bone substitute to the matrix was based on the lack of consensus regarding the benefit of this composite therapy as a ridge preservation procedure [2, 8, 9]. Moreover, the bone substitute may prevent the collapse of the membrane [13]. To our knowledge, such investigation using this combination of biomaterials has not been reported so far.

Materials and Methods

Design of the study

Ethical approval was obtained from the Ethical Committee of the “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania (No. 442/23.03.2012). The study was carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/1986 EEC). Two experimental dogs in good health, 12-month-old and weighing 11 and 13 kg, respectively, were used in the study. In both quadrants of the mandible, the distal sockets (resulted after the removal of the distal roots) of the second and forth premolars served as experimental sites for ridge preservation. Two sockets were left to heal naturally, three sockets were preserved with a collagen matrix (Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland) and fixed for 48 hours. Then, the samples were decalcified by standardized, controlled manufacturing processes without any cross-linking or chemical treatment. The collagen is extracted from veterinary certified pigs and is carefully purified to avoid antigenic reactions. Bio-Oss® is a bone substitute for regenerative dentistry having osteo-conductive properties. It consists of mineral granules size 0.25–1 mm without any organic component.

Surgical procedure

The surgical procedure followed well-established protocols. Briefly, the animals were anesthetized: administration of atropine sulphate (Nycomed®, Nycomed Austria GmbH, Linz, Austria) [0.04 mg/kg intramuscular (i.m.)] and diazepam (Diazepam®, Terapia SA, Cluj-Napoca, Romania) [0.2 mg/kg i.m.], injection of ketamine 10% i.m. (Ketamin®, ProduLab Pharma b.v., Raamsoonksveer, The Netherlands) (3 mg/kg), administration of isoflurane® 2% (Lunan Pharmaceutical Group Corporation, Shandong, China) plus oxygen mixture. Local anesthesia was performed with articaine 4% in solution with epinephrine 1:100 000 (Septanest®, Septodont, Quetigny, France). After that, the following steps were accomplished: grinding of the crowns of the mandibular second and forth premolars to their cervical third, endodontic treatment of mesial roots, restoration of the entrance of the pulp chamber on the remaining mesial roots.

The surgical procedures respected the following steps: elevation of buccal and lingual full thickness flaps, periodontal incision for flap mobilization, hemi-section of the premolars, extraction of the distal roots of the premolars. The flaps were retained with horizontal mattress and interrupted 4-0 resorbable suture (Vicryl®, Ethicon Inc., Johnson & Johnson, USA). The experimental sites were preserved before suturing the flaps with (a) the collagen matrix, or (b) with the collagen matrix plus the bone xenograft. All post-extraction sites were completely covered with the mobilized flaps to achieve a primary intention healing.

A strict regimen was administered after the surgeries: (1) daily observation of the animals for any clinical abnormality; (2) antibiotic administration for seven days (Dutaphen®; Wyeth-Lederle Pharma GmbH, Wien, Austria, 1 mL/day); (3) pain control (Dipyrone – Algocalmin®, Antibiotic, Iasi, Romania, 1 mL two times/day) after the surgery and on the following day; (4) a soft diet throughout the entire observation period; and (5) a plaque control regimen that included tooth cleaning twice a week.

Sampling procedure

After one month of healing, the samples were harvested following the current protocol. The jaw segments corresponding to the premolar sites that included the mesial root and the distal socket area were dissected using diamond burs and fixed in 10% buffered formalin solution.

Histological analysis

The animals were evaluated at the end of the study. All extraction sockets healed uneventfully; no dehiscence of the interventional sites was present during the observation period.

The samples were processed following routine histological procedures. Immediately after extraction, the samples were placed in 10% neutral buffered formalin and fixed for 48 hours. Then, the samples were decalcified
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in neutral 14% ethylenediaminetetraacetic acid (EDTA), for 20 days and dehydrated in progressive concentrations of ethanol (80% alcohol two times for 30 minutes; 95% alcohol two times for 30 minutes; and 100% alcohol two times for 30 minutes). The specimens were embedded in paraffin. Serial sections, 5 μm thick, in bucco-lingual direction, were prepared. Sections from the central and external areas of the sockets (mesial and distal parts) were harvested using a Microtom Gm BIT HN 310 (Germany) and were stained with Hematoxylin and Eosin (HE). The sections were fixed with DPX medium. The sections were examined by one author (CMM) by light microscopy (Leica DM 750, Germany) and were photographed with Leica ICC 50 HD (Germany) camera connected to the microscope.

For each preserved socket six sections in buccal–oral direction were sampled, two samples from mesial, central and distal part of the sockets, respectively. A total number of 48 sections from eight alveoli were finally considered for the histological analyses (12 for natural healing of post-extraction sockets, 18 for the matrix-preserved sockets, and 18 for the matrix plus bone substitute preserved sockets).

**Results**

The natural healing of the soft tissue covering the post-extraction sockets was mostly associated with the formation of a non-keratinized stratified squamous epithelium, which was highlighted by the presence of the nuclei of squamous cells in the superficial epithelial layers (Figures 1, 2a, and 3). A normal overall appearance of the component layers, epithelial and lamina propria could be reported (Figures 1 and 2). A discrete lymphocytic infiltrate was observed in lamina propria (Figure 2b). Rare parakeratinized zones (Figure 3) of the epithelium were observed alternating with non-keratinized ones at the level of the ridge mucosa.

![Figure 1 – Natural-healed socket: non-keratinized squamous epithelium (HE staining, ×200).](image1.jpg)

![Figure 2 – Natural-healed socket (HE staining, ×400): (a) Normal aspect of the epithelial layer; (b) Lymphocytic infiltrate in lamina propria.](image2.jpg)

![Figure 3 – Natural-healed socket: parakeratinized area of the epithelium (HE staining, ×400).](image3.jpg)

When the specimens from collagen matrix-preserved sockets were analyzed, the soft tissue revealed areas of parakeratinized epithelium alternating with keratinized ones (Figure 4, a and b). A thicker lamina propria was observed for these specimens than in the specimens harvested from the naturally-healed sockets (Figure 5a). Many collagen fibers randomly distributed and scattered eosinophilic fasciculi without attached fibrocytes were present in lamina propria (Figure 5, a and b). No signs of inflammation were tracked (Figure 5b).

An obviously thicker epithelial layer when compared with natural healing covered the sockets preserved with collagen matrix plus bone substitute. The entire soft tissue covering the ridges seemed to have a parakeratinized appearance, which was highlighted by the presence of the nuclei in the superficial epithelial layers and the obvious lack of the cell limits at this level (Figure 6a). Collagen...
fibers with a disorganized deposition were present in the lamina propria of all specimens and no inflammatory infiltrate appeared in any of them (Figure 6b). These findings were found in both external and central parts of the mucosa covering the former sockets. An interesting observation in the lamina propria was the predominately-fibrilar aspect of the connective tissue; on the direction of some collagen fibers, no attached fibrocytes could be seen suggesting the presence of some unresorbed debris from the collagen matrix (Figure 6b).

When the healing of the hard tissue of the unpreserved sockets was evaluated, a mature osseous structure was observed in the apical and central thirds of the former sockets demonstrating a normal aspect. Resting osteoblasts were present in these areas suggesting the completion of the post-extraction healing (Figure 7a). In the central third of the sockets, the lamellar bone lining the former alveolus was still present, showing a normal structure, with Haversian canals containing cells and vascular elements, proving thus the trophicity of the area (Figure 7b). A cancellous structure of the crestal part of the alveolar ridge displaying an immature aspect was observed, permitting a visible expansion of the covering soft tissue downwards (Figure 7, c and d).

**Figure 4** – Matrix-preserved socket (HE staining, ×400): (a) Keratinized epithelium; (b) Parakeratinized epithelium.

**Figure 5** – Matrix-preserved socket (HE staining, ×400): (a) Thick epithelial layer; (b) Absence of inflammatory infiltrate.

**Figure 6** – Socket preserved with the collagen matrix plus a bone substitute: (a) Parakeratinized epithelium (HE staining, ×400); (b) Disorganized collagen fibers in lamina propria (HE staining, ×200).
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Figure 7 – Naturally-healed socket: (a) Trabecular bone in the central part of the alveolus (osseous traveae with normal structure and areolae with a few marrow elements; (b) Lamellar bone in the central third of the alveolus; (c) Connective tissue bridging the bone structures in the crestal part of the alveolus; (d) Crestal connective tissue bridge – a closer view. HE staining: (a, b and d) ×200; (c) ×40.

The same healing aspects as those shown for naturally-healed sockets were observed in the apical and central parts of the alveolus when collagen matrix-preserved specimens were evaluated, namely, a normal, mature bone structure, highlighting the completion of bone healing (Figure 8a) and the presence of the lamellar bone delinating the former socket (Figure 8b). At the base of the socket, at the level of the former desmodontium, nervous fascicules could be marked out, some of them displaying discontinuity of the connective tissue capsule (perineurium) (Figure 8c). In the external third of the former sockets, the aspect of the trabecular bone was suggestive for a mature tissue hosting remodeling processes in which active osteoblasts, lining the osseous traveae, were responsible for the deposition of the new bone tissue (Figure 8d). A cancellous bone structure seemed to bridge the crestal margins of the buccal and lingual walls of the alveolar ridge with apparently no connective tissue interruptions (Figure 8e). A connective tissue rich in densely-packed fibers and possible membrane remnants could be observed in the profound parts of the alveolar mucosa closely covering the bone structure (Figure 8f).

Figure 8 – Matrix-preserved sockets (HE staining, ×200): (a) Trabecular bone with osseous traveae and areolae with few marrow elements; (b) Lamellar bone in the central third of the socket with a normal aspect.
Figure 8 (continued) – Matrix-preserved sockets: (c) Two intra-alveolar nerves – transversal and longitudinal sections; (d) Trabecular bone in the external third of the former socket containing active osteoblasts; (e) Trabecular bone facing the soft tissue of the ridge with no connective tissue interruption; (f) Deep region of the connective tissue of the ridge mucosa. HE staining: (c, d and f) ×200; (e) ×40.

Regarding the healing of the hard tissue in sockets preserved with collagen matrix plus bone substitute, a normal aspect of the trabecular bone neighboring the bovine bone particles was recorded, with the presence of areolae containing active osteoblasts and well-developed vascular structures (Figure 9a). In the entire alveolus, bovine bone particles were incorporated in a homogenous matrix resembling a bone-like structure with a very close contact between autogenic and bovine bone particles (Figure 9, b and c); an overall immature bone aspect could be remarked in the central and external thirds of the socket (Figure 9b). The collagen fibers connecting the bone and the composite structure formed by bovine particles and the interposed newly synthesized bone had a random distribution. Many fibrocytes were located on collagen fiber route (Figure 9d). A structure indicative for a more incipient osseous synthesis than in the other regions was present in the external third of the former alveolus. There were observed many active osteoblasts lining the peripheral parts of the bovine bone particles, osseous matrix and scarce osteocytes in osteoplasts (Figure 9f). The crestal aspect of the alveolar ridge was characterized by the presence of the trabecular bone with an immature appearance alternating with the composite structure bridging the distance between the buccal and the lingual bone walls; a discrete penetration of the covering soft tissue was observed (Figure 9e).

Figure 9 – Collagen matrix plus bone substitute-preserved socket: (a) Areola of trabecular bone containing many active osteoblasts and vascular structures; (b) Well-integrated bovine bone particles in the surrounding immature osseous tissue, in the alveolar central part. HE staining: (a) ×200; (b) ×40.
Discussion

The present study was designed in the context of the uncertain circumstances related to the best therapeutic option for ridge preservation. This study belongs to a large theme of research aiming to investigate the short- and long-term outcomes of some ridge preservation approaches. Using an animal model previously validated by our team [24, 27], we observed the pattern of the early healing of post-extraction sockets preserved with a collagen matrix with or without a bovine bone substitute and compared it with the naturally-healed sockets. The choice of the collagen matrix was based on the excellent reports related to its capacity to increase the quality of the soft tissues [17–19], but also on its possibility to extend the barrier function, due to its increased thickness, having in view that the use of two resorbable membranes prolonged the duration of the assembly’s integrity [28].

In our study, the healing pattern of the soft tissue covering both types of preserved ridges showed the presence of an obviously thicker epithelial layer in comparison with naturally-healed sockets. Moreover, the soft tissue over the preserved sockets contained mostly areas of parakeratinized epithelium alternating with keratinized ones while in naturally-healed sockets a non-keratinized epithelium predominated. The good outcome in terms of soft tissue formation observed by the present study may be due to the capability of the collagen matrix to increase the width and height of keratinized tissue, as demonstrated when used as a soft tissue substitute in root coverage surgeries [17–19, 29–31] or for ridge preservation [27]. The special design of the collagen matrix with a thick porous layer could induce a space-creating effect and favor clot formation [32, 33]. In specimens from preserved sockets, the fibrillar areas of the profound regions of the lamina propria that did not harbor attached fibrocytes, suggested the presence of some unresorbed debris from the collagen matrix. This was in agreement with other observations related to the prolongation of membrane integrity up to six months when a thicker device, such as a double-layer membrane approach, was used for guided tissue regeneration [28]. This important result could sustain the clinical preference for the collagen matrix over other devices such as collagen cross-linked membranes, which, despite having an increased biodurability, feature a diminished biocompatibility due to the lack of integration with the surrounding tissues [15, 34].

An interesting finding was the presence of a discrete but otherwise normal inflammatory infiltrate in the mucosa in specimens from naturally-healed sockets in comparison with the lack of inflammatory cells in specimens from preserved sockets. This is just another proof related to the extreme biocompatibility of the investigated collagen matrix.

Overall, the present study observed a better quality of the overlaying mucosa covering the ridges when the collagen matrix was used, in comparison with the naturally-healed sockets. The quality of the soft tissue consecutive
extractions is an important issue to be considered, as adequate dimensions of the mucosa covering the alveolar ridge are mandatory to obtain favorable long-term outcomes for dental implants. Recent research has provided information that the lack of keratinized mucosa around implants correlates with more plaque accumulation, soft tissue inflammation [35], mucosal recessions [36, 37] as well as loss of attachment [20]. Despite hundreds of studies developed on ridge preservation subject, very few have reported soft tissue-related outcomes [26, 27]. Ridge preservations should be focused on the approaches and materials capable not only to limit bone resorption but also to increase the dimensions in height and thickness of the ridge mucosa.

Regarding the hard-tissue healing in the apical third of the former sockets, the same normal, mature bone structure highlighting the completion of bone healing was recorded for all the three types of post-extraction approaches. While in naturally-healed and collagen matrix-preserved specimens the central third of the sockets showed a mature bone structure, in collagen matrix plus bone substitute-preserved sockets an immature appearance was observed in the aforementioned area. In the external third of the former sockets, the bone displayed immature characteristics in naturally-healed and in collagen matrix plus bone substitute-preserved sockets. A different aspect of this region was recorded for matrix-preserved alveoli with the trabecular bone, suggestive for mature tissue hosting remodeling processes. It seemed that the use of a collagen matrix alone protected the healing phenomena, allowing the development of more mature external bone structures four weeks after extraction. However, until being indicated as a single therapeutic approach for routine clinical use, further studies should evaluate its barrier function.

On the other hand, a delay of osseous formation associated with the presence of bovine bone particles could be assumed in comparison with the use of the matrix alone. This is somewhat different from our previous results on early healing [24], when an increased osseous deposition was suggested to be associated with the use of the collagen matrix plus another bovine bone substitute (BioOss Collagen®, Geistlich Pharma AG, Wolhusen, Switzerland) in comparison with the use of double-layer membranes. The different composition of the former biomaterial containing a mixture of bone particles and collagen might be responsible for this finding. However, it could be presumed that bovine bone particles would stimulate the mineralization of the neighboring collagen fibrous structure after four weeks.

Regarding the matrix plus bovine bone-preserved sockets, our results are concordant with other observations reporting no gaps at the bone–particle interface with the bone being always in close contact with the particles. Other authors observed a more uniform distribution of bovine bone particles in the mass of cancellous bone [38].

We noticed more advanced healing phenomena developed in naturally-healed sockets in comparison with other studies using the same animal model, which reported the presence of the provisional matrix in the central portions of the sockets alternating with mineralized tissue mostly formed by woven bone and bone marrow [39, 40]. A woven bone structure was reported to fill all the inside of the sockets with the incipient formation of lamellar bone four weeks after extraction [3].

Our study described at the base of the former sockets, preserved with the collagen matrix, the presence of nervous fibers. In many areas, the connective tissue capsule surrounding the nerves displayed a discontinuous aspect, suggestive for a traumatic lesion consecutive to tooth extraction, and in the meantime, a slower reparative process of nervous fibers in comparison with the osseous tissue. To our knowledge, no classical study, using the dog model and observing the histological pattern of the healing after ridge preservation, reported data on re-innervation [25, 39, 41–43]. Tooth extraction results in damage to a large number of sensory nerve fibers, but the pattern of healing after this damage is incompletely understood. It was suggested that multiple extractions in cats [44] and humans [45, 46] could lead to the development of traumatic neuromas that may, in some circumstances, play a role in the genesis of chronic oro-facial pain. Six months after tooth extraction in cats, proprioceptive fibers were still present within the bone in the areas where the teeth had previously been and may reinnervate new tissue, suggesting that the extraction did not necessarily result in a neuronal loss [47]. Three-dimensional reconstruction and measurement of local innervation density showed progressive changes in the pattern of innervation during healing of the extraction sockets in ferrets [48]. The innervation previously contained within the confines of the periodontal ligament proliferated and gradually invaded the central region of the socket. The peripheral innervation density peaked at one-month post-extraction, while the central zone innervation density gradually increased in a three-month period. After three months, the innervation started to arrange into longitudinal nerve trunks running up through the former socket from the apical to the coronal aspect [48]. The fate of nervous structures and periodontal ligaments would be an issue for further investigations.

The entrance of all the preserved sockets was “closed” by a cancellous bone bridge that connected the buccal and lingual crests with apparently no or minimal connective tissue intrusion. The expansion of the covering soft tissue through superficial cancellous bone was obvious in naturally-healed specimens. This could highlight the enhanced barrier function of the surgical place provided by the collagen matrix and its possible stimulating effect on bone formation.

Our study aimed to completely cover the post-extraction ridges with flaps in order to obtain a primary intention healing and to ensure a good development of the healing phenomena as well as to protect the matrix from early degradation. Some studies have reported less remodeling with flapless extraction procedures [49], but recent data observed that flapped surgical techniques demonstrated a significantly lesser horizontal resorption of the sockets, when compared to flapless surgeries [11]. Moreover, flapped surgical approaches also allow the insertion of the membranes that further protect the post-extraction sites.

One of the drawbacks of the present research is the low number of animals but based on the current results further investigations are planned. We would aim to
observe late healing phenomena developing in post-extraction sockets when the same biomaterials are used providing information that could be important to gain their clinical acceptance.

Conclusions

A thick, well-keratinized or parakeratinized mucosa covered the ridges preserved with the collagen matrix alone or associated with a bovine bone substitute. The use of the collagen matrix could be a clinical option for preserving post-extraction ridges especially when an improvement in soft tissue dimension and quality is desired. Some differences in hard tissue development and distribution were identified between the three types of healing of post-extraction sockets. The preservation using the collagen matrix alone allowed the development of more mature and continuous external bone structures four weeks after extraction. The use of the collagen matrix seems to be an interesting ridge preservation option but only after obtaining further information on its barrier function. The presently used bovine bone substitute seemed to delay hard tissue development after tooth extraction.

Conflict of interests

The authors declare that they have no conflict of interests.

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