Dokkyo Journal of Medical Sciences
48 (03) (2021)

Use of Adipogenic Stem Cells in Treatment of Oronasal Fistulas in Dogs

Mikhail A. Sergeev[1*], Dina A. Azizova[2], Rais G. Hafizov[2], Aigul R. Hairutdinova[2], Ilsur G. Galimzyanov[1], Olga I. Shorkina[1], Anastasia N. Valeeva[1], Nazhiya V. Shamsutdinova[1], Samat R. Yusupov[1], Zoya G. Churina[1]

Kazan State Academy of Veterinary Medicine by N.E. Bauman, Sibirsky tract 35, Kazan city, 420029, Russia[1]
Kazan (Volga Region) Federal University, Kremlevskaya street 18, Kazan, Russia, 420008, Russia[2]

Corresponding Author: 1*

ABSTRACT— Application of stromal cells in dentistry opens wide possibilities for the use of cell technologies in maxillofacial surgery, periodontology, and implantology. Tooth decay, alveolar periodontitis, parodontopathies, fractures of jaw bones and dental crowns are common in dogs and lead to oronasal and oroantral fistulas in neglected cases. Adipose tissue stem cells (ATSCs) have recently been of special interest to biologists and physicians. ATSCs are able to differentiate into bone, cartilaginous, adipose, muscle, nerve tissue cells, and into vessel wall cells (endothelial cells and satellite cells). The study subjects were dogs belonging to Kazan city inhabitants. During the clinical study, oronasal fistulas in the upper alveolar ridge area were diagnosed in all animals. We performed operations on guided tissue regeneration in the oroantral fistula area with the single- step fine tissue sculpting. Allogeneic mesenchymal stem cells in combination with titanium nickelide granules (Nitigran) were used as osteoinductive material. The complex of osteoconductive materials and allogeneic MSCs mesenchymal stem cells, introduced into the fistular canal, not only provides complete elimination of fenestration between the nasal and the oral cavities, but also stimulates full osteanaphysis.

KEYWORDS: Dog, oronasal fistula, dog, allogeneic mesenchymal stem cells

Received March 8, 2021； accepted March 24, 2021

1. Introduction

Teeth play an important role in vital fucntions of small domestic animals. However, in most cases, cat and dog owners do not realize that dental care in animals is not less important than in humans. Veterinary clinics in our country mostly use such treatment method as tooth extraction in practice, which is not always justified [1], [2]. According to veterinarians, neglection of therapeutic technique in dentistry is connected with the absence of technical and methodical equipment of veterinary offices and clinics, and scientifically grounded recommendations for use of dental restoration materials for these purposes [3- 5]. Tooth decay, alveolar periodontitis, parodontopathies, fractures of jaw bones and dental crowns are common in dogs and lead to bone defects of alveolar ridges, expressed as oronasal and oroantral fistulas in neglected cases [6], [7].

Bioengineering studies of stem cells properties and their possible use in surgery are actively carried out in human and veterinary medicine. Mesenchymal stem cells are able to transform almost into any type of body cells. Adipose stem cells have recently been of special interest to biologists and physicians [8- 10]. In view of this, the goal of the study was set: development and

assessment of the efficiency of the method of surgical treatment of oronasal fistulas in dogs with the use of adipogenic allogeneic mesenchymal stem cells and the carrier from Nitigran material.

2. Materials and Methods

The study subjects were dogs belonging to Kazan city inhabitants: German shepherd, female at the age of 8, chow-chow, female at the age of 5, and standard flat coated wiener dog, male at the age of 10. During the clinical study, oronasal fistulas in the upper alveolar ridge area were diagnosed in all animals. Osteoplasty in the fistulous tract area was performed with the use of general potency narcosis. Allogeneic mesenchymal stem cells (MSCs) were obtained from the epiploon during abdominal incision from the chow-chow female.

To stimulate cell differentiation in osteogenic direction, they were cultivated on Osteocyte medium, and in chondrogenic, on Chondrocyte medium (Gibco, USA).

To establish the fact of differentiation, cell structures were fixed and dyed on day 21. To establish mineralization, Von Kossa reaction was used, and to establish the fact of chondrogenic differentiation, dying with Alcian Blue was used.

Biocompatible fine-grain porous titanium nickelide (Nitigran) with the pore size from 0.1 to 1000 μm and the mesh titanium nickelide shape memory membrane, obtained by self-propagating high-temperature synthesis in Tomsk Research and Development Institute for Shape Memory Materials and Implants in Medicine, were sterilized in the hot-air oven at 180^0C for 1 hour prior to use. The cell treatment medication was prepared immediately prior to transplantation. The cell suspension was mixed with Nitigran granules, placed on the Bioplast Dent bioplastic membrane, soaked in canine blood, and covered with the mesh titanium nickelide membrane [11].

X-ray study of the upper alveolar ridge in dogs was performed in oblique and lateral view and frontal view at initial attendance of ill animals, immediately after the fistulous tract closure surgery, and 30 days after it on the equipment Arman 9L5, on Kodac radiographic film. After the oronasal fistula closure surgery in dogs, their general health condition and appetite were estimated; body temperature, pulse and respiration rate were measured daily. Oral cavity examination was performed, suture consistency and integrity, together with color, swelling and soreness of soft tissues were estimated (Fig. 1).

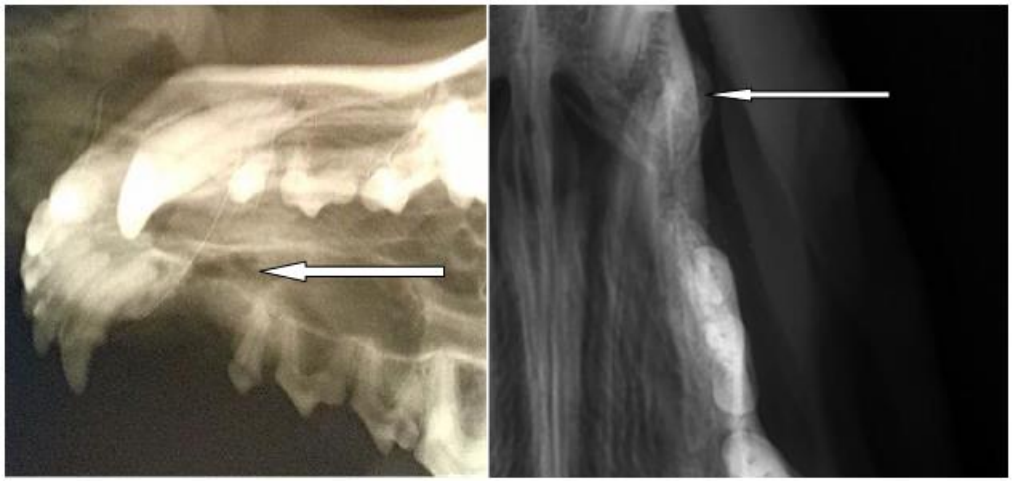

Figure 1. Dental X-rays of the dog's upper jaw bone in oblique and lateral view and frontal view (the fistula localization is indicated with an arrow)

Canine blood was taken from the dogs' cephalic vein into vacutainer tubes, both prior to the surgery and in 24 hours, 7,14 and 30 days after it. Total protein, total calcium, inorganic phosphorus concentrations, and activity of alkaline phosphatase in serum were determined on Piccolo Express 25 biochemical analyzer.

3. Results and Discussion

According to the studies, oronasal fistulas in all dogs were formed during 1,5-2 months after the removal of eyeteeth. In the course of the oral cavity examination, defects of the mucous membrane were detected in the area of absent eyeteeth along the transitory fold with length of 1-1.2 cm and width of 0.2 -0.4 cm (Fig.2).

At canal probing, a bulbous-end probe penetrated into the nasal cavity for a depth from 3.8 to 7.2 cm. depending on the dog's body size.

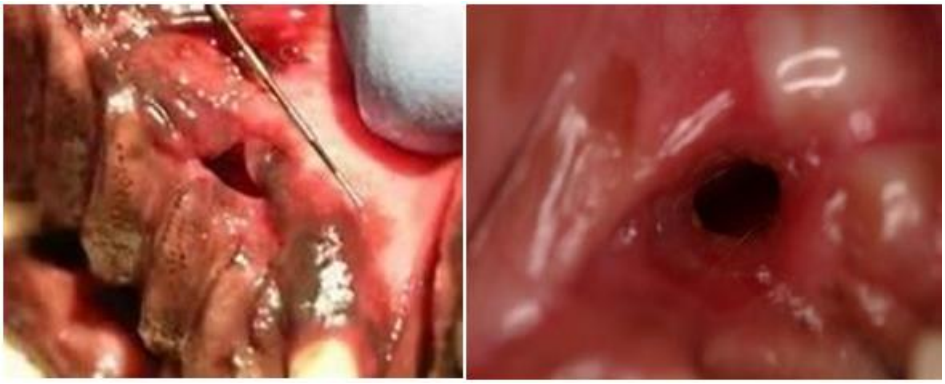

Figure 2. Fistula appearance from the oral cavity side

Animals were fixed on the surgery table in lateral position, the fistula was cleaned from residues of food masses, sanitated with the furacillin solution 1:5000, the mucous membrane around the fistulous tract external orifice was twice treated with the 1% povidone iodine solution. Removal of the eyetooth root fragments, the fistulous tract revision, the resection of granulating tissue and epithelium throughout the depth was performed. Changes sites of the upper jaw cortical plate, forming the fistula walls, were removed with cylindrical fissure dental burs, continious cooling provided, with the sterile 0.9% sodium chloride solution. The suspension of mesenchymal stem cells (6 mln) was mixed with Nitigran granules, placed on the bioplastic membrane, and wrapped in the mesh titanium nickelide membrane.

The material complex was introduced with the surgical forceps into the fistulous tract canal, filling the upper alveolar ridge defect with it (Fig. 3).

At the distance of 3-4 mm medially and distally in relation to the fistula, parallel discissions of the gum and the periosteal coverage to the site of the gum transfer into the lip mucous membrane were made; then, the mucoperiosteal flap was prepared and pulled on the bone defect. The wound edges were sutured with interrupted knotty sutures using the polyglycolic thread No. 4 (Fig. 4).

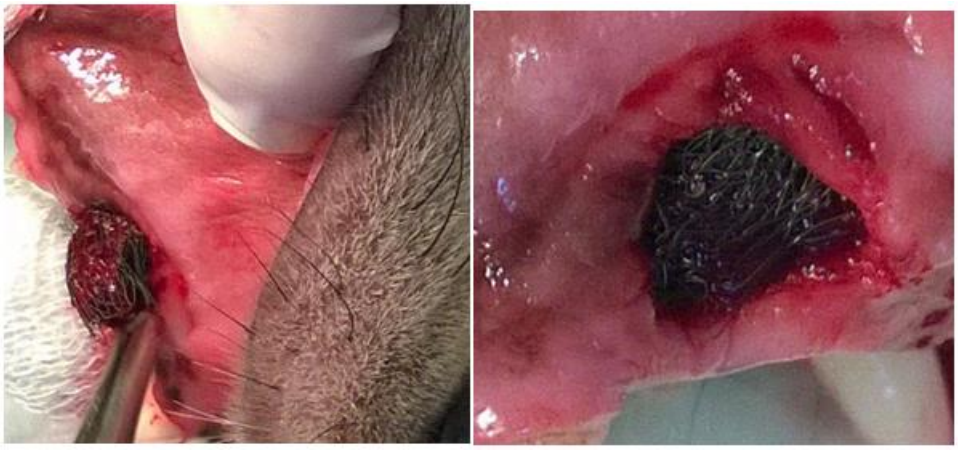

Figure 3. Filling the fistula canal with the material complex

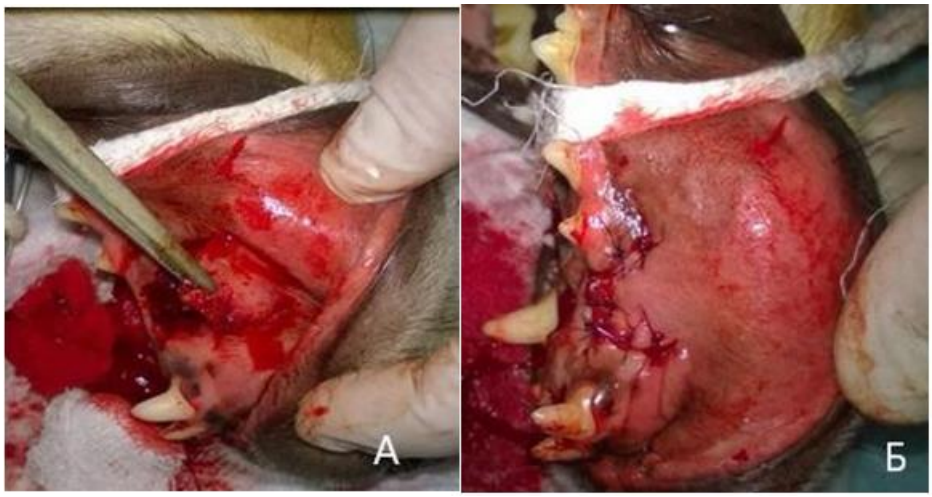

Figure 4. Oronasal fistula plastic reconstruction with soft tissues.

A) Mucoperiosteal flap separation B) Sutured wound of the mucous membrane after the plastic surgery

Antibiotic ceftriaxone was prescribed as antimicrobial therapy 2 b.d.s. i.m. in the dose of 50 mg/kg of body weight per day for 7 days. Pet owners were recommended to give the dogs only food of soft consistency, and to sanitate the oral cavity with the furacillin solution 1:5000 after each feeding for 2 weeks. Throughout the postoperative period, general health condition of all dogs was satisfactory. During the first day, the animals demonstrated slight bleeding from a nostrile, corresponding to the fistula localization, that stopped spontaneously. All the dogs demonstrated food affectability already in 12-24 hours after the surgery. Hyperemia and swelling of the mucous membrane of the wound edges preserved for one week after the surgery, sutures were fixed well.

Changes in basic clinical indicators of all the animals status post had the similar dynamics. In 24 hours after the surgery, body temperature decrease and slight heart and respiration rate increase were observed. On day 7 and further on all observation terms, indicators characterizing the clinical status of the animals conformed to the physiological standard values. In serum study, activity of alkaline phosphatase increased more than 9 times in each dog in 24 hours after the surgery, then, decreased by day 7, then increased repeatedly and remained exceeding the normal value during one month.

Due to change of ostease activity in blood, total calcium concentration also changed. During the first two weeks after the surgery, this indicator has been gradually increasing by 14 % in average compared to the initial level, and remained increased within one month (Table).

Table: Biochemical indicators of canine serum

Indicators	Ill animal	Study period				
		Before surgery	After surgery, in			
			24 hours	7 days	14 days	30 days
Total protein, g/l	German shepherd	60.2	58.9	64.0	68.0	67.8
	Chow-chow	67.4	65.4	67.3	68.1	66.9
	Flat coated wiener dog	68.8	65.3	67.9	68.0	67.8
Alkaline phosphatase, U/l	German shepherd	28.8	212.5	256.8	254.6	121.1
	Chow-chow	22.0	214.5	35.0	125.4	124.6
	Flat coated wiener dog	22.7	214.1	44.0	123.1	123.8
Total calcium, mmol/l	German shepherd	2.24	2.7	2.62	2.64	2.56
	Chow-chow	2.5	2.89	2.66	2.7	2.8
	Flat coated wiener dog	2.3	2.7	2.78	2.79	2.67
Inorganic phosphorus, mmol/l	German shepherd	1.67	1.54	1.36	1.56	1.6
	Chow-chow	2.0	1.44	1.41	1.8	1.86
	Flat coated wiener dog	1.70	1.32	1.57	1.54	1.72

On dental X-rays of the dogs, performed immediately after the surgery, Nitigran granules and the titanium nickelide membrane were visualized as X-ray contrast structures in the fistulous tract area. At this, precise localization of the material in the eyetooth alveole and the alveolar ridge defect zone, without penetration into the nasal cavity, was observed. In 30 days, the dental X-rays of all dogs status post revealed the formed internal cortical plate that was separating the eyetooth alveole from the nasal cavity, with its structure not differing from the surrounding skin areas in terms of radio density. An oronasal fistula canal was absent. In addition, Nitigran and the titanium nickelide shape memory membrane was fully integrated into the newly formed tissues (Fig. 5,6).

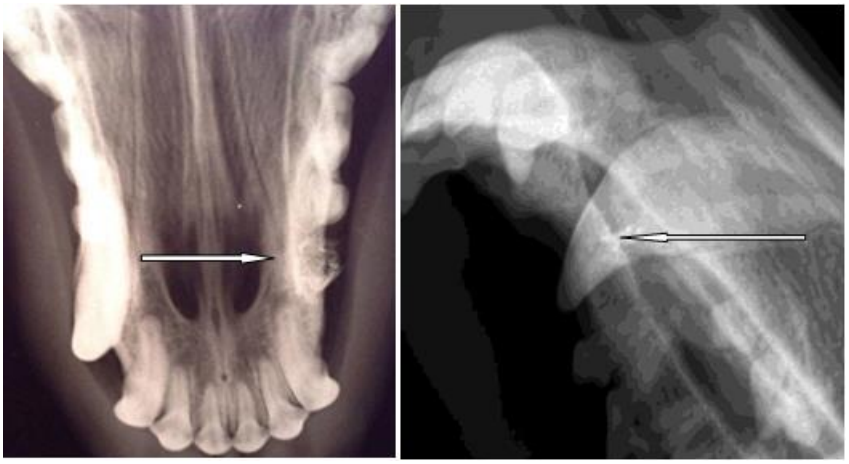

Figure 5. Dental X-rays of German shepherd's upper jaw in 30 days after the oronasal fistula closure surgery

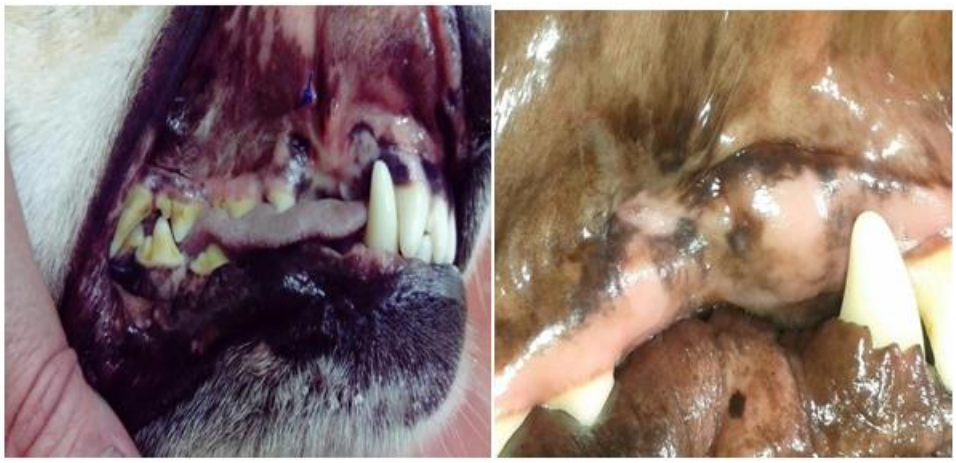

Figure 6. Complete healing of the mucous membrane by primary intention in the area of oronasal fistula closure surgery in dogs

A) Chow-chow; B) Wiener dog

Healing of the mucous membrane wound took place by primary intention in all dogs by week 3 after the surgery.

4. Conclusion

Thus, the study findings demonstrate the reasonability of use of the proposed osteoplasty method for bone defect removal. The proposed method of surgical treatment of oronasal fistulas in dogs is characterized by low invasiveness and requires no considerable material cost. The complex of osteoconductive materials and adipogenic allogeneic mesenchymal stem cells, introduced into the fistular canal, not only provides complete elimination of fenestration between the nasal and the oral cavities, but also stimulates full osteanaphysis. MSCs, obtained from canine adipose tissue, have the capacity for osteogenic and chondrogenic differentiation in culture on dedicated media. The increase in activity of alkaline phosphatase in canine serum, related to the increase in activity of osteoblasts, is detected within one month after the closure of oronasal fistulas.

5. References

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