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Research Article

Osteogenic potential of Gingival Mesenchymal Stem Cells on Chitosan scaffold for Treatment of Peri-implant Defects (An Experimental Study)

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Abstract

Background: Implant therapy is now considered an acceptable successful way to replace hopeless teeth. the purpose of this study was to Evaluate the use of gingival mesenchymal stem cell (GMSC) on chitosan scaffold in the treatment of peri-implant defects. **Method:** A total of ten mongral dogs age ranging between 1 to 2 years old weight from 20 to 25kg were used in the current study. Dogs were scheduled for first premolars extraction one on each side and immediate replacement by immediate implant at mesial socket with creation of peri-implant defect at distal socket. Sites were randomly assigned in a split mouth design into two groups: control group in which the defects were filled with chitosan only, intervention group in which the defects were filled by Gingival Mesenchymal Stem Cells (GMSCs) carried on chitosan scaffold. Five dogs will be sacrificed 1month and the others will be sacrificed at 3 months post operation. Bone density was measured around each implant at defect sides at baseline (implant insertion), 1 month and 3 months. **Results** IBM SPSS 28 for windows software was used for the analysis, there was a significant difference in bone density change, as bone density change was higher in intervention group, p-value > 0.05 at one month but there was no statistically significant difference observed between the two groups at three Oral medicine, Diagnosis and periodontology department Faculty of Dentistry Minia University Egypt **Conclusion:**using gingival mesenchymal stem cells in treatment of peri-implant defects gives better results in shorter duration, but same results can be reached with chitosan alone in longer duration and less costs. More studies with larger sample size are recommended.

Keywords: Gingival Mesenchymal Stem Cells, Peri-implant Defects, Chitosan scaffold

Introduction

Implant therapy is now considered an acceptable successful way to replace hopeless teeth [(1)]. Immediate implantation has many advantages like proper implant placement, decrease time needed for to receive prosthesis and patient satisfying with function and esthetics at time of implantation [(2)].

However, the main challenge of immediate post extraction implants is significant alveolar bone loss due to periodontal disease, abnormalities, traumatic injury, or physiological bone resorption. Many modalities are used nowadays to deal with these challenges. One of them the technology of tissue engineering that has proven to be a promising

therapy for bone regeneration with the establishment of an artificial biomaterial containing regenerating-competent cells and osteogenic growth factors achievable [3].

Today, using of stem cells is considered as a mainstream strategy for periodontal therapy, particularly for complete regeneration of the periodontal complex, which implies not only the reconstruction of appropriate alveolar bone but also the induction of cementogenesis along the root surfaces with the oriented insertion of newly formed periodontal tissue [4]. Regarding dental originated sources, gingival mesenchymal stem cells (GMSCs) considered more interesting alternatives to the other dental MSCs as they are much easier to get from the clinically resected gingival tissues. Therefore, it is of great concern to prove the multiple differentiation potentials of GMSCs for possible tissue engineering applications [5].

For tissue engineering, scaffolds are needed to provide a three-dimensional microenvironment to accommodate cells and guide their adhesion, growth, and subsequent differentiation. A plethora of materials have been developed for tissue engineering approaches, namely natural and synthetic polymers, metals, ceramics, and composites, which have been fabricated into porous scaffolds, microparticles, sponges, meshes, nanofibers, and gels.

Natural polymers are preferred over synthetic or metallic materials, since they have better biocompatibility and ability to degrade in vivo without releasing toxic substances [6]. One of these natural polymers commonly used is chitosan which is biocompatible and biodegradable and is currently used with other polymers in a variety of tissue engineering applications.

Many studies revealed that using autogenous or xenogenous (human) mesenchymal stem cell MSC to treat peri-implant defects gives higher results in bone formation and bone implant contact than using synthetic bone graft alone.

Also, treatment of peri-implant defects with bone marrow derived MSC and bone marrow mononuclear cells that had undergone ex vivo osteogenic differentiation prior to clinical use[7] resulted in higher new bone apposition than scaffolds alone.

Materials and Method

A total of ten mongral dogs age ranging between 1 to 2 years old weight from 20 to 25kg were used in this study. All experiments were conducted in the animal house of the Faculty of Medicine, Cairo University, Egypt according to the recommendations and approval of the Ethics Committee on animal's experimentation of the Faculty of Medicine, Cairo University.

1- **Isolation of gingival mesenchymal stem cell (GMSC):**
   Gingival sample will be obtained by resecting a small piece of gingiva to prepare gingival mesenchymal stem cell (GMSC). G-MSCs will be obtained from the healthy gingival collars around partially impacted third molars. Cells' isolation and culture will be done as formerly described [8].
2- **Biodegradable scaffold synthesis:** A biodegradable composite made of Chitosan (Ch) will be generated by a simple molding method as previously described [9].
3- Animal preparation: The animal model will be prepared as previously described by Boix and colleagues [10].
4- Surgical procedures: dogs will be anesthetized by intramuscular injection of ketamine (10 mg/kg) and xylazine (4 mg/kg), measures will be taken to minimize pain to the dogs. Under sterile conditions, the first mandibular premolar teeth will be extracted bilaterally. Immediately, distal bone defects adjacent to the mesial socket 6 mm in height, 4 mm in the bucco-lingual direction, and 5 mm in the mesio-distal direction were created in the mandible bone. Then titanium implants were installed into the mesial area of the bone defect in each side. The primary stability was assessed.
5- Defects were randomly divided into 2 groups, control group in which defects were filled with chitosan scaffold alone and intervention group in which defects

were filled with GMSCs carried on chitosan scaffold. Five dogs were sacrificed 1 month, and the others were sacrificed at 3 months post operation.

6- **Radiological evaluation** was done by taking periapical radiographs at baseline, 1month and 3 months after implant placement according to time of scarification. Bone density was measured using Digora software.

7- Data were collected, tabulated and statistically examined

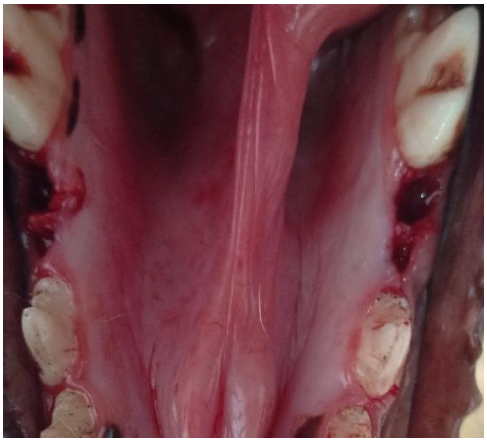

Fig.1 after extraction of 1st premolar bilaterally

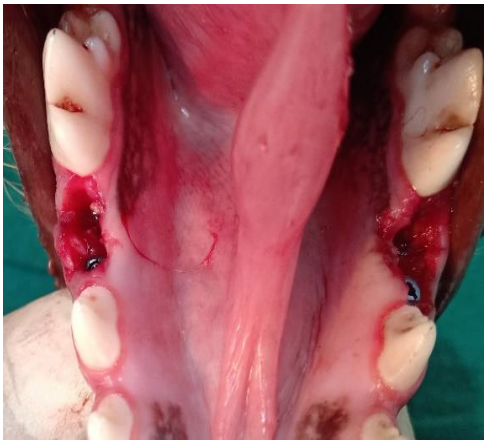

fig.2 after implant placement bilaterally

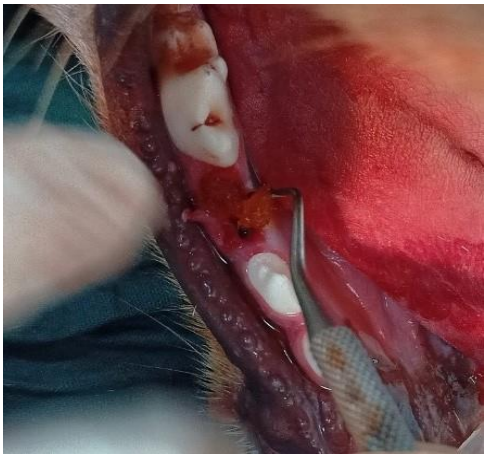

Fig.3 placement of stem cell on chitosan scaffold

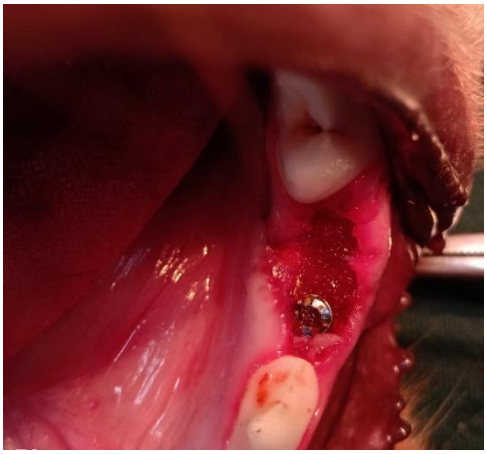

fig.4 placement of chitosan scaffold

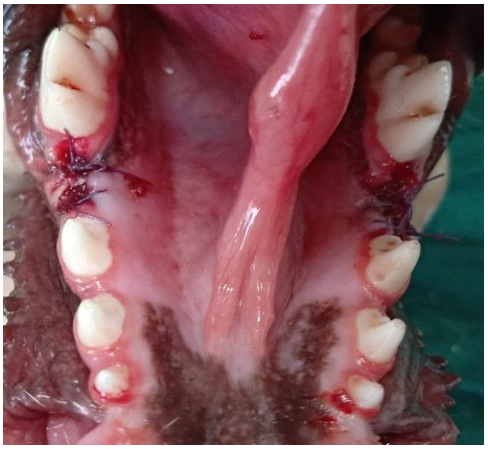

Fig. suturing bilaterally

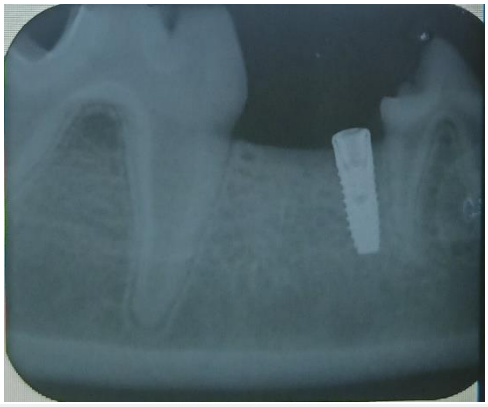

Fig. 6 Right side after one month

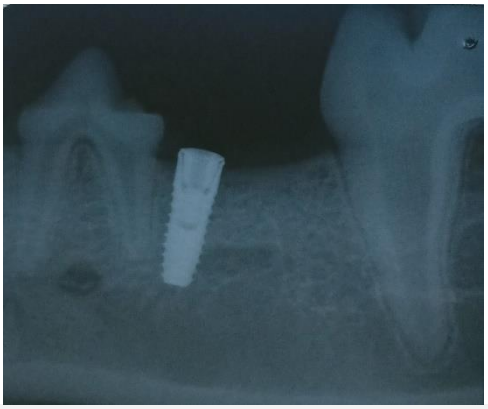

Fig. 7 Left side after one month

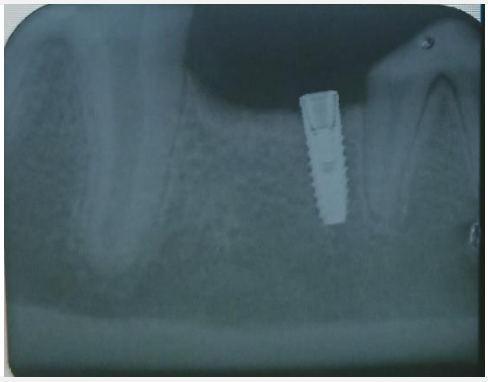

Fig. 8 Right side three month

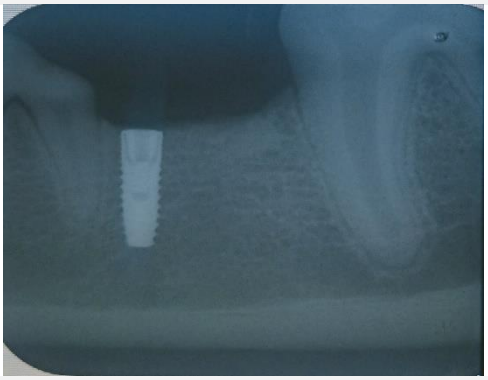

Fig. 9 Left side three month

Results

Paired sample t-test was done to compare bone density at baseline and after one and three months between control group and intervention group.

	Intervention group		Control group		Mean difference	P-value
	Mean	**SD**	**Mean**	**SD**		
Bone density change	58.73	11	19.71	2.6	39.02	*0.003

There was a significant difference in bone density change, as bone density change was higher in intervention group (mean= 58.73, SD= 11.00) than in control group (mean= 19.71, SD=2.60), p-value= 0.003.

	Intervention group		control group		Mean difference	P-value
	Mean	**SD**	**Mean**	**SD**		
Bone density change	41.14	6.61	44.29	5.45	-3.15	0.343

No statistically significant difference was observed between the two groups regarding bone density change.

Discussion

Stem-cell-based therapies aiming at regeneration of bone defects have been a promising alternative for clinical trial[11]. As novel postnatal stem cells, GMSCs have been paid extensive attention for their therapeutic potential in regenerative medicine [12].

GMSCs can be easily isolated from human gingival tissue which is usually discarded as biological waste in the clinic and proliferate rapidly in vitro to meet the transplantation requirement for cell amount.

The contribution of GMSCs to bone regeneration was confirmed by radiologic assessment of the bone density in the present study. As we found, at 1- and 3-months post transplantation, the newly formed bone in intervention group was significantly higher than that in control group at one month and it was also higher at 3 months at intervention than control but without significance.

In addition to the osteogenenic potential of GMSCs to promote the new bone forming, another possible explanation responsible for the therapeutic effects of GMSCs on bone injuries was the transplanted GMSCs triggered the endogenous MSCs recruitment which is known to be crucial for successful bone repair [13], though the mechanisms of MSCs recruitment to the injury sites were unclear.

Conclusion

Stem-cell-based therapies have been a promising alternative for bone regeneration. Selection of appropriate donor cell types plays an important role in successful cell transplantation. The present study provides evidence that systemically transplanted GMSCs can not only home to the peri-implant defect but also promote bone regeneration. Given the basic characteristics of MSCs and advantages such as ease of isolation, high proliferation capacity, uniformly homogenous property, and so on, GMSCs are considered as an ideal candidate cell resource for cell-based therapies. Future studies using large animal numbers and longer duration are needed to assess the long term safety and efficacy of GMSCs for bone regeneration.

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