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Role of Stem Cells in Transport Distraction Osteogenesis for Reconstruction of Mandibular Defects after Segmental Mandibulectomy

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ABSTRACT

Purpose: to restore the continuity and functions of the mandible after segmental mandibulectomy for mandibular defect reconstruction by using a technique of transport distraction osteogenesis with stem cells injection. **Subjects and methods**: Clinical study was conducted on eight patients suffering from mandibular deficiency after tumor resection. All steps of manufacturing of the transport distractor, tumor resection and segmental mandibulectomy of transport disc were guided by Stereolithographic (STL) model. Stem cells sample was prepared to be injected along the soft callus at the end of the activation period. Patients were followed up postoperative clinically every week and radiographically by Panoramic x-ray and computed tomography (CT) at immediate postoperatively, then by panoramic x-ray monthly along the periods of distraction and finally by multi slice CT at the end of the consolidation to evaluate the time consuming for treatment. **Results:** seven cases out of eight cases recorded excellent distraction regenerate and excellent soft tissue coverage and one case out of these eight cases in the control group failed at the first week of activation. Multi slice CT showed a significantly higher value in the mean of the regenerated bone density at the end of the consolidation (1163.76±111.38) in the study group than the control group (747.67±268.6). **Conclusion:** From our study, stem cells injection in the distraction regenerate for reconstruction of mandibular defect cases achieved short consolidation period and high regenerated bone density.

KEYWORDS

STL model, Transport Distraction osteogenesis, Stem cells injection.

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INTRODUCTION

The repair of large maxillary and mandibular bony defects caused by trauma, tumors or congenital malformation stills a reconstructive challenge. Maxillo-mandibular complex restoration enhances facial aesthetics and functions such as speech, mastication, swallowing and breathing[1,2].

Segmental mandibular defects considered the most challenging problems for reconstruction, especially when involved curvilinear parts. Although the micro vascular bone free flaps are the most successful method for reconstruction of the large defects, however they still sustain obstacles such as the morbidity associated with long operating and hospitalization time make it excluded in some cases. So less invasive reconstructive techniques are needed in selected patients [3,4].

At the traditional transport distraction osteogenesis, the transport disc is obtained by cutting the original bone in one side with keeping the soft tissue and periosteum attached. However, for patients with severe bony defects, the gaining of optimal transport disc is not always possible[10].

The prolonged treatment of DO increases the incidence of infection and appliance breakage, autologous mesenchymal stem application to the site of distraction accelerates the process of DO and shortens the treatment time. With the aim of mandibular reconstruction, it becomes easy to restore the continuity and functions of the mandible through transport distraction osteogenesis after segmental mandibulectomy[11,12].

SUBJECTS AND METHODS

Study design and population a prospective comparative study was designed and conducted on eight patients suffered benign mandibular tumors and need segmental mandibular resection. They were selected from the outpatient clinic of the Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine for Girls, Al-Azhar University, Al Zhraa University Hospital, and Nasr City for health insurance Hospital, from November 2019 to April 2021. The patients were randomly and equally divided into two groups. Group 1: segmental mandibular resection was reconstructed with Transport distraction osteogenesis using computer designed custom made transport disc distractor. Group 2: the mandible was reconstructed with TDO using computer designed custom made transport disc distractor and stem cell injection, at the beginning of the consolidation period. Selection of patients was done according to inclusion criteria; Patients with benign or aggressive benign tumors indicated for segmental mandibular resection, presence of preoperative and postoperative computed CT, complete clinical and pathologic records, and highly motivated and cooperated patients[1]. The exclusion criteria included, lesions that are not indicated for mandibular resection, cases with malignant tumors, medical conditions that could interfere with the healing process such as nutritional deficiency, uncontrolled diabetes, chemotherapy, radiotherapy... etc. and inability of the patient to return in follow up visits[7].

In accordance with the Declaration of Helsinki, a written informed consent was taken from all patients, and the study was approved by local ethics review committee of the Faculty of Dental Medicine at Al-Azhar University for Girls, with ethic code REC-SU-21-04.

Surgical protocol

Pre-surgical preparation

Preoperative clinical and radiographic (panorama & CT) evaluations were performed to all patients to evaluate the extent of the mandibular lesion and defect[13].

Stereolithographic model

The radiographic data was then converted into the Standard Tessellation Language format (STL format) and sent to a 3D printer, computer

aided manufacturing (CAM) system, generating a sterolitho graphic (STL) model for patient mandible. The expected resection margins were drawn on the model, and then the 2.4 mm reconstruction plate (Leibinger plates, Stryker Howmedica GmbH) was manually contoured and adapted with pliers by the same operator until an optimal fitting was achieved[14].

Designing of the custom made transport distractor

Drawing the design of the transport distractor on the STL model as well as marking the area of the future transport disc. Custom made transport disc distractor was designed according to the location and extension of the defect and the site of the future transport disc. Each distractor has three plates: one plate on the transport disc and the two others were placed on the two sides of the mandible, which aid in the fixation of the distractor on the mandible. The distractor has an attached guide that looks like a hook to maintain the parallelism between the reconstruction plate and distractor during the activation of the distractor, as well as achieving stability of the transport disc.[15]

Surgical management

Preoperative patient preparation: a) Good oral hygiene measures, extraction of any loose teeth or remaining roots, which may cause any problem after surgery such as inflammation or infection. b) Standard laboratory investigations including: complete blood count (CBC) Hemoglobin A1C (HbA1c) test, liver and kidney function, Prothrombin time (PT), Partial Thromboplastin Time (PTT), and the International Normalized Ratio (INR) and Thyroid function analysis. c) Chest x-ray& Corona virus analysis. d) Under local anesthesia, incisional biopsy was taken for histopathologic analysis, and the treatment plan was chosen according to the histopathologic report. e) Patients were hospitalized one or two days before surgery.

Surgical steps (Fig.1):

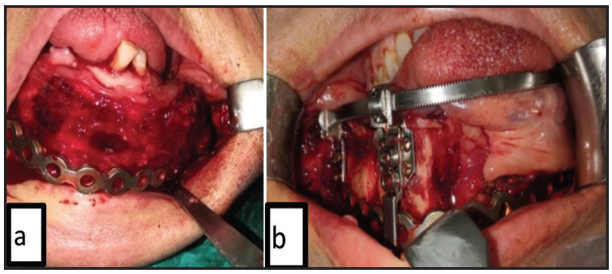

Figure (1) Showing a) temporary placement of the reconstruction plate before lesion resection. b) proper placement and fixation of the transport distractor plates, one at the transport disc and another distal to it

All surgical procedures were performed under general anesthesia, by the same oral and maxillofacial team. All patients underwent segmental mandibular resection reconstruction with the pre-bent titanium reconstruction plates. Before surgical intervention, arch bars were applied for patients with remaining dentition in the upper and lower jaws. Via an intraoral approach, the buccal and lingual flaps were raised, and the entire mandible was exposed according to the extension of the lesion. Intermaxillary fixation (IMF) was performed to preserve the occlusion of the unaffected side. Before resection, the reconstruction plate was inserted and temporarily fixed in the correct position with 2.7mm screws (11mm length) on the proximal and distal segments. Osteotomy lines were marked on the mandible to determine the area of lesion resection as well as the future transport disc. The transport distractor was also inserted, and the location of plates screws were marked on the bone using a bone drill to facilitate the final distractor placement. The tumor was resected with safety margin according to the planned osteotomy lines using oscillating saws, chisels, and osteotomes. The lesion was submitted for pathologic examination. The transport disc was created by resecting about 2cm of normal bony block, close to the bony defect. Proper fixation of the transport distractor by screws of 2mm diameter. After completing the osteotomy,

the distractor was activated, with the attached guide in place, for few millimeters to ensure complete separation of the transport disc, and to determine if there will be friction or obstruction of transport disc movement due to the presence of the attached guide during its movement toward the anterior curved area of the mandible. Finally, the transport disc was repositioned back to the zero position. Closure of the vestibular incision was carried out. The surgical site was inspected for any bone debris and irrigated with normal saline. Lingually: suturing of the genioglossus and mylohyoid muscles to the reconstruction plate anteriorly, to preserve the integrity of the mouth floor and the tongue movement. Buccally: suturing the buccal mucosa without mentalis muscle to avoid obstruction of the transport disc movement during the period of activation. The buccal and lingual flaps were sutured together with continuous resorbable suture (3/0 vicryl)[5].

Postoperative care & medication

Postoperative care: A pressure band was applied at the chin area for 48 hours after surgery. The patient was asked to preserve good oral hygiene by using mouth wash diluted in saline. The patient was kept on liquid diet (to avoid chewing). Unasyn (Ampicilin/Sulbactam) 1500mg intravenous every 12 hours for 5 days. Decadron (Dexamethasone) 8 mg/6 hours for the first day and 4mg /6 hours for the second day and Voltaren 75mg (Declofenac sodium, 75 mg ampoules, produced by NOVARTIS PHARMA S.A.E. Cairo under license from Novartis Pharma AG., Basel, Switzerland) IM injection were prescribed. The oral cavity was irrigated once/day with normal saline for the first postoperative week. Chlorohexidine(Chlorhexidine HCL 0.125%, Cairo A.R.E., A.D.C.O.) mouth rinses were prescribed for daily use. Application of ice packs to the cheek and chin regions for 10 minutes every 30 minutes for the first 24 hours. Hot fomentation for 10 minutes every 30 minutes was performed on the next day of surgery for 7 days.

Postoperative clinical and radiographic evaluation[14].

Postoperative clinical evaluation: All patients were followed up daily for 5days postoperatively, then weekly during the activation and consolidation periods to evaluate the following parameters: wound healing, suture breakdown, and dehiscence, transport disc exposure, checking for presence or absence of pain, numbness, swelling, infection, hematoma, or bleeding.

Postoperative radiographic evaluation

a) An orthopantomogram (OPG) was taken immediately postoperative to ensure the proper placement of the reconstruction plate and the transport distractor.

b) CT scan was requested for all patients: at 5th day of the operation (the first day of activation period) to assess the proper position of the transport distractor in relation to the reconstruction plate.

Distraction protocol

Activation period: Activation of the transport distractor was started on the 5th postoperative. The distraction rate was about 0.75mm / day at a rhythm once daily. This process was carried out by activating the device one full turn using the activation wrench/ key. First activation was done by the medical team, then by the parents or by the patient himself. CT was performed 1 week after the beginning of device activation and at the end of consolidation period. Patients were followed up weekly until the end of activation period, when the transport disc reached the docking site. Radiographic follow-up was performed to assess callus formation[7].

Stem cells sample preparation and injection (study group):[16](fig.2)

Stem cells sample was prepared and injected on the first day of consolidation period.

By a bone marrow trocar (gauge 13), a puncture was made to penetrate the anterior superior iliac spine with a watch wind movement. Aspiration of 20 ml bone marrow was obtained in a heparin-treated 50 ml syringe. Repositioning of the trocar was done for each 10 ml in the same cortical access hole to access different areas of cancellous bone marrow. Aspirate was then centrifuged to separate the BMMNS(bone marrow mono nuclear cells) using the density gradient separation method, in which Ficoll paque plus (GE Health care, Bukinghamshire,UK) bottle was shaken / agitated several times to insure thorough mixing and then 3 ml of the media was added to the centrifuge tube. Dilution of The bone marrow aspirate (1:1) with saline and then carefully layered on to Ficoll gradient and then centrifuged at 3000rpm for 15 minutes at room temperature. After centrifugation, aspiration of the middle cloudy layer containing the mononuclear cells was done carefully by a sterile tube using a sterile syringe. At the end of activation, the transport disc distractor was removed under general anesthesia and then stem cells sample was injected along the length of the soft callus in study group patients. This is followed by incisions closure.

Figure (2) Showing the Penetration of the bone marrow trocar

Consolidation period: When the distractor reached the end of the mandibular defect, the distractor activation was stopped to start the consolidation period.

Removal of the transport distractor: the distractor was removed at the end of the consolidation under general anesthesia (after detection of complete consolidation of the formed soft callus by CT).

Multi-slice CT was requested after distractor removal, to measure the height and width of the newly formed bone and compare these measures with the adjacent original bone in both control and study groups[3].

Postoperative follow-up [3]: The patients were evaluated clinically and radiographically by multi-slice CT.

Clinically: The devices were found to be well tolerated by all patients who were able to perform their normal daily activities without great discomfort except one case due to the remote area of the transport disc. All patients noticed a change in their appearance as treatment progressed and they were satisfied with the final results.

Radiograghically: Multi slice CT shown evidence of consolidation for the newly formed soft callus in two groups at the end of the consolidation period.

Statistical analysis:

Data were presented as percentage values and were analyzed using Fisher's exact test. Numerical data were presented as standard deviation (SD) and means values. They were examined for normality by checking the data distribution, and using Shapiro-Wilk test. Data showed parametric distribution so they were analyzed using independent t-test. The level of significance was set at $p \leq 0.05$. Statistical analysis was recorded with R statistical analysis software version 4.1.1 for Windows (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.).

RESULTS

Demographic data: Eight patients with mandibular benign tumor or already had resected mandible due to mandibular benign tumor who completed the follow up were included in the statistical analysis of this study.

Multi slice CT postoperative outcomes: (table 1, and figure 3)

The mean value of density of the regenerated bone was calculated at the end of the consolidation period. The statistical analysis revealed that the mean value of density in study group showed a significantly greater value than the control group (p=0.04). Also, the consolidation period was recorded which was measured by weeks. It was (18.67±4.16) in the control group and (7.25±0.96) in the study group as shown in figure (3) . The mean of activation period was measured by weeks. It was (14.00±8.72) in the control group and (10.75±3.59) in the study group. So, it had no significant statistical difference in these two groups. The mean of the regenerated bone height was measured by mm for each group. It was (22.43±5.20) in the control group and (19.28±1.42) in the study group. So, it had no significant statistical difference in these two groups. The mean of regenerated bone length was measured by mm. It was (33.92±17.27) in the control group and (31.31±6.87) in study group. So, it had no significant statistical difference in these two groups. The mean of the transport disc length measured by mm. It was (19.20±0.92) in the control group and in the study (19.18±0.45) in the study group. So, it had no significant statistical difference in these two group. The finding of these measures is that the regenerated bone length is independent on the transport disc length.

Clinical postoperative outcome:

Seven cases out of eight cases recorded excellent distraction regenerate and excellent soft tissue coverage. Clinically, one case in the control group recorded fracture distractor due to aggressive activation by patient after one month of activation. Wound dehiscence was found in 33.3% of the control group. The lingual inclination of the transport disc was found in single case in each group. Edema was negative in each group. Tongue space narrowing was reported single case in each group. Loosening or loss of transport disc teeth was reported in single case in each group. Numbness was reported in single case in each group. So, there was no statistically significant difference in clinical outcome between the control and study groups.

Table (1)

Parameter	(mean±SD)		p-value
	Control	Study	
Regenerated bone height	22.43±5.20	19.28±1.42	**0.288ns**
Regenerated bone width	8.14±1.39	5.18±0.39	**0.009***
Regenerated bone density	747.67±268.59	1163.76±111.38	**0.035***
Defect length	47.57±3.15	38.04±8.08	**0.116ns**
Transport disc length	19.20±0.92	19.18±0.45	**0.976ns**
Regenerated bone length	33.92±17.27	31.31±6.87	**0.790ns**

**; significant (p ≤ 0.05) ns; non-significant (p>0.05)*

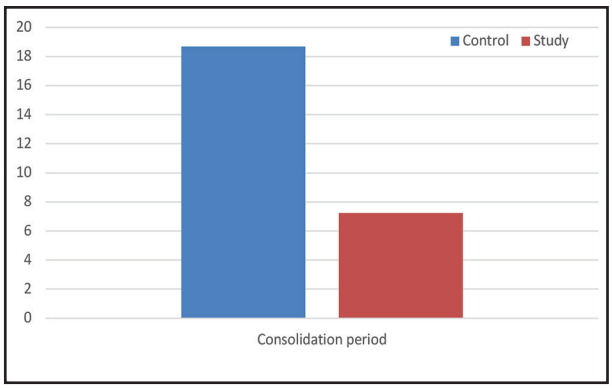

Figure (3) *Bar chart showing average Consolidation period (weeks) for different groups*

DISCUSSION

The mandibular defects reconstruction will restore the facial contour, esthetic, speech, masticatory function, and swallowing. Consequently, these patients' quality of life will be improved[17,18]. So, it is critical to select an appropriate reconstruction technique for each case of mandibular resection, in order to provide acceptable outcomes for these patients[19].

Distraction osteogenesis was proven by some authors to be successful with high success rate in reconstruction of large mandibular defects[20]. Recent researches in tissue engineering documented usage of stem cells for bone and soft tissue regeneration, which achieved successful results in DOG[21]. Kitoh was the first one using bone marrow aspirate for gaining MSCs in the distracted soft callus to reduce time of distracted bone healing[22,23]. This encouraged our study for large mandibular defects reconstruction by distraction osteogenesis with stem cell injection.

Statistical analysis of multi slice CT images in our study revealed that the regenerated bone density shown statistical significance at the end of the consolidation period for both control and study group which was measured by HU. Similar findings were observed by Zongyang sun et al who stated that, proper gap obliteration, higher mineral content and faster mineral apposition occurred in cases received stem cell injection in the distraction gap[24]. Distractor fracture was found in one case in the control group after one month of activation which may be due to aggressive dealing with the distractor by the patient. Wound dehiscence was found in 33.3% of the control group which may be friable nature of the soft tissue coverage around the distractor, in addition to the bad oral hygiene may contribute to this complication. The lingual inclination of the transport disc was found in single case in each group regarding to the action of the mylohyoid muscles that act on the movable transport disc specially when moving in curved area on the mandible as the canine area leading to lingual inclination of this transport disc taking the soft callus with it and causing some sort of tongue space closure. Most of these postoperative complications were also mentioned by Madah et al. as he divided the complication of the distraction osteogenesis in to distractor or device related and non-device related. Device related as fracture of the distractor and non-device related as wound dehiscence.[25,26]

Fortunately, these results indicate that, the stem cell injection has high successful rate in cases of mandibular distraction osteogenesis.

CONCLUSION

Stem cells injection in the distraction regenerate for reconstruction of mandibular defect cases achieved short consolidation period and high regenerated bone density.

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RECOMMENDATION

Recently, using stem cells in cases of distraction osteogenesis it is very successful.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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