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Characteristics of Alveolar Bone Marrow Cells from Patients Undergoing Dental Extractions or Dental Implant Therapy

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University

by

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Abstract

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Abstract: Alveolar bone marrow stromal cells (aBMSCs) play important roles in craniofacial wound healing. To establish an easy, efficient and reliable method to harvest aBMSCs, we compared three different methods: extraction socket aspiration, osteotomy aspiration and bone core digestion. Samples of aBMSC were collected from two groups of subjects. Group 1 (dental extraction): after dental extraction, 22.5-gauge needles were used to collect 0.5-1cc marrow aspirate. Group 2 (dental implant): during implant surgeries, bone core and 0.5-1cc marrow aspirate were obtained from the osteotomy. Samples were cultured in petri dishes and attached cells were expanded. The population doubling time (PDT), surface markers, and osteogenic differentiation potential of these cells were studied. In total 12 subjects were enrolled in the

study. The success rates of generating aBMSCs from extraction socket aspiration, osteotomy aspiration and bone core digestion were 42.8% (3/7), 40% (2/5) and 80% (4/5), respectively. Cells from extraction socket aspiration had the fastest proliferation rate among the three sample types, followed by bone core and osteotomy aspiration, as shown in PDTs and DNA fold changes. After isolation and expansion, all the aBMSCs expressed high levels of CD 73, CD90, and CD105, however, the expression of CD146 varied among the cells. Cells derived from bone core had the highest ALP activity after osteogenic induction, followed by cells from osteotomy aspiration, and then extraction aspiration. Taken together, bone core samples obtained during implant surgery is a more reliable source for generating aBMSCs and aBMSCs harvested from different methods may have different characteristics.

Key words: Stem cell, alveolar bone marrow stromal cells, regeneration, implant therapy.

Introduction

Untreated periodontitis may lead to progressive loss of clinical attachment, followed by destruction of the periodontal ligament and the supporting bone around the periodontium which may ultimately lead to tooth loss[1]. Tooth loss is a severe public problem, especially in the elderly population, that causes loss of chewing function, malnutrition, esthetic problems and decreased life quality. It is estimated that over 240 million people in the industrialized world are missing one or more teeth, and 40% of the Western population has lost at least one tooth[2]. Based on the National Health and Nutrition Examination Survey (NHANES) in 2004, seniors over 65 years of age lose an average of 10 teeth per person, and 27.7% of them have no remaining teeth[2]. Dental implants have become a standard of care for the replacement of missing teeth and over 1 million implants placed annually in the United States. Although the long term success rate of dental implants is over 90%[3–6], the clinical outcome of implant therapy is impacted by many systemic conditions, such as diabetes[7], smoking[8] and osteoporosis[9,10], and local factors that include insufficient alveolar bone volume[11].

Regenerative medicine strives to repair organs and/or tissues affected by chronic disease[12]. Stems cells are applied in regenerative medicine and disease therapeutics, however, the function and nature of mesenchymal stem cells (MSCs) have gone through a number of paradigm shifts[13,14]. Currently, it is believed that MSCs contribute to tissue regeneration through two important functions; first, the ability to differentiate into distinct end-stage cell types that include bone, cartilage, muscle, tendons, ligaments, fat, dermis, and other connective tissues; second, the ability to initiate a broad spectrum of bioactive molecules that promote tissue regeneration in injured sites[15]. In regenerative medicine, embryonic stem cells (ESCs), tissue specific progenitor

stem cells (TSPSCs), mesenchymal stem cells (MSCs), umbilical cord stem cells (UCSCs), bone marrow stem cells (BMSCs), and induced pluripotent stem cells (iPSCs) have been widely studied [12]. ESCs have been shown to treat spinal cord injuries[16], promote macular defect recovery and vision restoration[17], regenerate liver tissue after injuries[18], and restore damaged cartilage in athletes[19]. TSPSCs have been used to regenerate cochlear[20], ischemic myocardium[21], and goblet mucosa in intestine[22]. UCSCs have been applied to the repair of injured tendons and cartilage[23], the treatment of Hodgkin's lymphoma and other cancers[24], and the restoration of beta cell function in diabetes[25]. BMSCs have been utilized to treat AIDS[26], neurodegenerative diseases[27], and aplastic anaemia[28]. iPSCs have been shown to be promising in the treatment of diabetes, COPD and liver degeneration[29]. A shift of viewpoint acknowledges that MSCs affect damaged tissue repair through paracrine or cell-to-cell communication to stimulate host cells[13].

MSCs were traditionally isolated from the iliac crest bone marrow, which was first reported by Friedenstein in 1976[30]. This method is generally adopted and widely used in regenerative medicine. However, the procedure involving iliac crest bone marrow aspiration makes routine isolation of MSC for craniofacial regenerative therapy difficult, especially in dental offices[31]. In addition, studies have suggested that site specific differences exist in MSCs derived from iliac crest compared to orofacial (maxilla and mandible) origins[31]. For craniofacial regeneration, cells from craniofacial tissues may be more beneficial compared to those from iliac crest[32].

Currently MSCs can be obtained from several different dental tissues[33]. Periodontal ligament is a fibrous and vascular tissue that contains progenitor cells that have features seen in mesenchymal stem cells[34]. MSCs can be harvested from periodontal ligament tissue of surgically extracted third molars[35]. Human dental pulp, apical papilla, and dental follicle are also potential sources for MSCs[33]. Dental tissue derived MSCs can promote craniofacial wound healing including

periodontal tissue regeneration. For example, periodontal ligament progenitors were demonstrated to enhance bony defect regeneration in animal models[36], and improve clinical parameters in humans with deep intrabony defects[37]. Dental pulp stem cells were seen to improve mandibular bone defect repair after 3rd molar extraction when delivered in a collagen sponge[38].

MSCs derived from alveolar bone (aBMSCs) has emerged as another important stem cell population for regenerative dentistry and implant therapy. Such stem cells can be achieved through the use of bone cores and/or marrow aspiration during dental extractions or implant therapy[39]. aBMSCs have a better accessibility than other dental MSCs and are considered the primary cells responsible for extraction socket healing and implant osseointegration. Therefore, better understanding of aBMSCs may provide valuable information regarding important aspects of craniofacial and dental wound healing.

A recent paper by Mason et al. described the standardization and safety of aBMSC isolation[40]. They isolated aBMSCs by alveolar bone marrow aspiration (~0.5cc) from 45 patients. Results from in vitro and in vivo experiments clearly demonstrated the reliability of using small volume aspiration to extract aBMSCs. However, they only collected samples from implant preparation sites, which significantly limits the application of this technique to implant patients only. Tooth extraction is a much more widely performed procedure in dentistry. It has been reported that aBMSCs may be isolated by bone marrow aspiration from 3rd molar extraction sites[31], however, it is unknown if this would be as efficient when applied to other extraction sites. It is also not clear whether bone marrow aspiration from extraction sites will be as effective as other technique such as bone core harvesting.

Although the use of MSCs in regenerative medicine is well documented in the literature, research pertaining to the use of aBMSCs in periodontal regeneration is limited. Therefore, the cellular characteristics of aBMSCs derived from aspiration of the bone marrow from an alveolar post extraction sockets are largely unknown.

The aims of this study were to extract mesenchymal stem cells from alveolar bone and evaluate their regenerative potential based on their osteogenic differentiation potential, population doubling time, surface marker characteristics and to investigate which method was more predictable in isolating MSCs from alveolar ridges. Once a reliable method is established, new studies can be developed to investigate the difference in MSCs derived from patients with different conditions such as diabetes and smoking.

Methods

Clinical procedures and sample collection

Approval for the study was granted by Virginia Commonwealth University Institutional Review Board (HM20013027). Patients in the Graduate Periodontics department who received routine dental extractions or dental implant treatment were screened by their primary provider. Patients that qualified for the study based on a checklist of inclusion and exclusion criteria (Figure 1) and were willing to participate the study were consented by the study coordinator. The patient then received either a dental extraction or implant placement by their primary provider using standard operating procedures.

The following protocols were followed to collect samples during the surgical procedures:

Group 1 (dental extraction): Following extraction of the tooth, the clinician inserted a 22.5-gauge needle connected to a heparinized 1cc syringe into the extraction socket, and approximately 0.5-1 cc of marrow aspirate (blood) was obtained. The clinician then continued with the surgery and post-surgical management based on the clinical situation.

Group 2 (dental implant): After elevation of a gingival flap at the position where the implant was planned to be placed, a bone core of 2×5 mm was harvested with a trephine bur as the step of initial osteotomy drilling. The bone core was stored in sterile saline before sending it the lab for analyses. Next, a 22.5-gauge needle connected to a 1cc heparinized syringe was inserted into the marrow space, and approximately 0.5-1 cc of marrow aspirate was obtained.

Cell culture

Alveolar bone marrow tissue samples were re-suspended in cold minimum essential alpha medium (αMEM; Gibco, Carlsbad, CA, USA) and centrifuged at 600 g for 10 min at room temperature. The supernatant was removed and the cell pellet was re-suspended in 5 mL aMEM with 15% fetal bovine serum (FBS; Gibco). The cell suspensions were then transferred to T-25 tissue culture flasks and allowed to sit undisturbed without media change for 5 days in a 37°C humidified tissue culture incubator at 5% CO2. Non-adherent cells were removed following 5 days in culture, and medium was changed to aMEM-10% FBS and changed every 2 to 3 days thereafter. Once adherent cells reached 80% to 90% (approximately 10-14 days), the aBMSCs were then collected and subcultured up to passage 3. Human BMSCs from iliac crest were purchased from RoosterBio (Frederick, MD, USA) and cultured in expansion media until they were ready to in vitro experiments. Human gingival fibroblasts (HGFs) were primary cells from one donor. They were a courtesy of Dr. William Giannobile in University of Michigan and Dr. Martha Somerman from NIDCR, and maintained in DMEM with supplements of 10% FBS and 1% Penicillin-Streptomycin. During the osteogenic experiments, same media were used for all the cells including hBMSCs and HGFs.

Population doubling time (PDT)

In order to evaluate the aBMSC proliferation and expansion rates in vitro, population doubling times were obtained for the samples. The average PDT was calculated between passage 1 (P1) and passage 2 (P2). An online website (https://doubling-time.com/compute.php) was used, which uses the following formula:

$$DoublingTime = \frac{duration * \log(2)}{\log(FinalConcentration) - log(InitalConcentration)}$$

Initial concentration = the seeding cell number

Final concentration = the cell number in the flask at the time of harvest.

The unit of duration is hour.

Flow cytometry

Flow cytometry was performed on samples to evaluate the expression of MSC markers according to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell Therapy (ISCT). CD 73, CD 90, CD 105, CD 146, and CD 45 MSC markers were used. aBMSCs were harvested from T75 flasks by 0.25% Trypsin, transferred into tubes, washed with DPBS, and incubated with blocking solution. The cells were then incubated with specific antibodies conjugated with a fluorochrome or isotype control antibodies for 30-45 minutes. Cells were washed extensively at each step. For fluorochrome compensation, antibodies were added to UltraComp eBeads (eBioscience) according to manufacturer's protocol. Analysis was performed on BD FACSAria™ II High-Speed Cell Sorter in VCU flow cytometry core. The list of antibodies included:

- PE conjugated anti human CD45 antibody, R&D MSC Marker kit (FMC002)
- Brilliant Violet 421 conjugated anti human CD73 antibody, #344007, Biolegends, San Diego, CA, USA
- APC conjugated anti human CD90 antibody, R&D MSC Marker kit (FMC002)
- PE/Cy7 conjugated anti human CD105 antibody, #323217, Biolegends, San Diego, CA, USA

- Brilliant Violet 711 conjugated anti human CD146 antibody, #323217, Biolegends, San Diego, CA, USA
- PE/Cy7 conjugated anti mouse IgG1 k antibody, #400125, Biolegends, San Diego, CA, USA
- APC conjugated anti mouse IgG2 antibody, R&D MSC Marker kit (FMC002)
- PE conjugated anti mouse IgG1 antibody, R&D MSC Marker kit (FMC002)

Osteogenic potential

The osteogenic potential of the samples was evaluated by the level of alkaline phosphatase (ALP) activity. Briefly, cells from different donors were cultured in 24-well plates with growth media for 24 hours, at which time the media were changed. Full media (DMEM with 10% FBS, 1% APS) was added to half of the plates. Osteogenic media (full media with the supplements of 50 μg/ml ascorbic acid, 10 mM β-glycerophosphate, 10 nMdexamethasone) was added to the other half of the plates. Media were changed every 3-4 days. At day 0, 3, 7 and 14 days, cells were harvested with 200 ul 0.05% Triton X-100. Total cell DNA was measured by Picogreen assay (Promega) and protein content were quantified by Pierce BCA protein assay (Thermoscientific). The ALP activities of cell lysates were then measured as a function of p-nitrophenol hydrolysis from p-nitrophenylphosphate at pH 10.2. The results were normalized to the total protein contents.

Statistical analysis

This was a pilot study to test the feasibility of different techniques, and therefore we were not able to perform statistical analysis on some of the experiments such as measuring PDTs. For

other in vitro experiment, only one donor was selected from each group. Statistical analysis was performed based on the results of 4 technical replicates of each cell type. The results were expressed as means ± standard error of the mean (SEM), and graphs were prepared using Graph Pad Prism 7.02 (Graph Pad Software, CA, USA). One-way analysis of variance (ANOVA) was conducted, followed by Tukey's multiple-comparison test. P-value smaller than 0.05 was considered to be significance. P-values were indicated using asterisks as follows: * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$), **** ($p<0.0001$). Statistical analysis was not performed in the cell surface marker experiment because only one donor was selected in each cell type and there was no technical replicate.

Patient selection

Inclusion Criteria

- Age range: 18 years or older
- Sex: Male and female
- Patients follow the study procedures and instructions
- Patients read, understood and signed an informed consent form
- Two groups of patients are recruited:
    - Patients in Group 1 require dental extractions.
    - Patients in Group 2 have one or more than one missing tooth on maxillary or mandibular arch and is having implant(s) to restore missing tooth/teeth

Exclusion Criteria

- Patients under 18 years of age
- Female patients who are pregnant
- Patients with prior radiation treatment, bone metastasis or other skeletal malignancy
- Periodontal abscess
- Patients with transmittable disease transmittable by blood
- Immediate Implant Placement
- Limited English Proficiency patients

Figure 1: Inclusion and Exclusion Criteria for patient selection

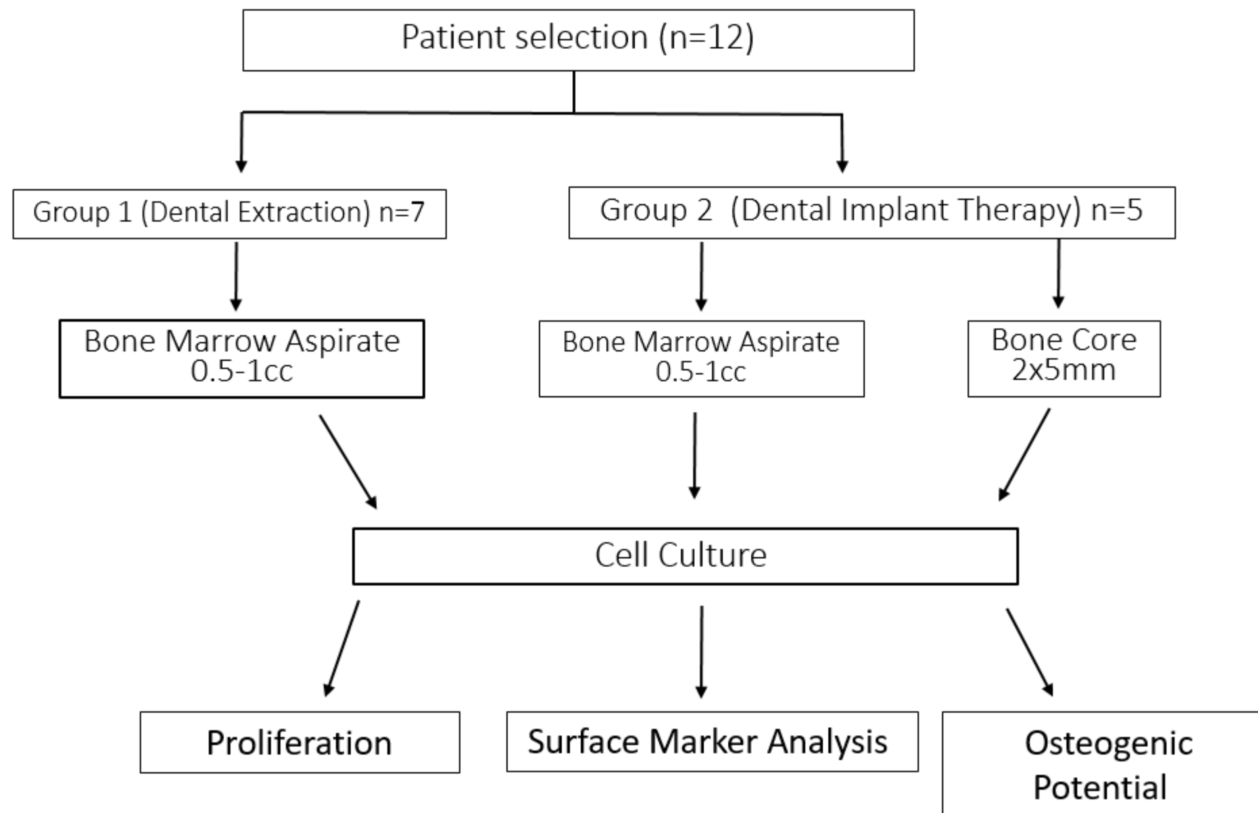


Figure 2: Flowchart summarizing patient selection and treatment groups.

ID	Gender	Age	Max/mand (tooth number)	Ext Aspiration	Population doubling time (PDT)/hours	Frozen vials	Implant Aspiration	Population doubling time (PDT)/hours	Frozen vials	Implant Bone Core	Population doubling time (PDT)/hours	Frozen vials
#001	M	70	Max #2	Yes	40.82	P1: 4 vials; P2: 10 vials						
#002	M	40	Max #9	Yes	No colonies							
#003	F	55	Max #3	Yes	46.42	P1: 5 vials; P2: 6 vials						
#004	M	45	Man #22,27				Yes	52.98	P1: 1 vials; P2: 12 vials	Yes	46.42	P1: 7 vials; P2: 6 vials
#005	F	66	Man #21	Yes	No colonies							
#006	M	76	Max #5				Yes	56.47	P1: 5 vials; P2: 7 vials	Contaminated		
#007	F	55	Man# 19,29,30				Yes	No colonies		Yes	55.98	P1: 2 vials; P2: 12 vials
#008	M	66	Max (2,3)				Yes	Didn't grow much	P1: 0 vial; P2: 2 vials	Yes	47.49	P1: 2 vials; P2: 8 vials
#009	F	37	32	Yes	No colonies							
#010	F	61	#23 and #27				Yes	No colonies/ contaminated		Yes	47.97	P1: 3 vials; P2: 9 vials
#011	M	35	#30	Yes	No colonies/ contaminated							
#012	F	34	#19	Yes	Successful, but PDT is not available	---						

Table 1: Summary of 3 different harvesting techniques

Results

Success rates of generating aBMSCs from different tissue sources

Bone marrow aspiration samples from extraction sites were collected from 7 subjects and, of these, 3 were able to generate aBMSCs (**Table 1** and **Figure 2**). Additionally, in 5 subjects who were receiving implant surgeries, bone marrow aspiration samples were collected from osteotomy sites and obtained the bone cores. aBMSCs were able to be generated from 3 marrow aspiration samples and from 4 bone cores. These cells had similar morphological characteristics, which were fibroblastic-like, and spindle-shaped. All of these cells were able to be expanded to passage 2 except for one osteotomy aspiration sample. Therefore, the success rates of generating aBMSCs from extraction aspiration, osteotomy aspiration and bone core were 42.8% (3/7), 40% (2/5) and 80% (4/5), respectively. Bone core samples obtained during implant surgery appeared to be the most reliable source for generating aBMSCs.

Cell proliferation capability

Cell proliferation capability was further assessed by calculating the PDT and a DNA assay. The average PDTs for samples from extraction aspiration, osteotomy aspiration and bone core were 43.62 h, 54.72 h and 49.46 h, respectively (**Table 1**). Although no statistical analysis was performed due to the small sample size (only 2 samples from osteotomy aspiration), it appeared that the proliferation rate of aBMSCs derived from osteotomy aspiration was slower than other cells. We selected one subject from each sample type and measured the changes in DNA content after expansion. Similarly, we found that cells from extraction aspiration had the fastest proliferation rate among the three sample types, followed by bone core and osteotomy aspiration (**Figure 3** and **Figure 4**). Similar results were seen both in growth medium (NT) and osteogenic

medium (OM). In this experiment, we also used BMSCs from long bone and gingival fibroblasts (HGFs) as controls. Interestingly, aBMSCs appeared to grow faster than long bone BMSCs, but slower than HGFs (**Figure 4**).

MSC characterization by surface markers

We also identified the cell surface markers by fluorescence-activated cell sorting (FACS) in each of the three types of samples. Following isolation and cell expansion (at passage 3), aBMSCs derived from extraction aspiration, osteotomy aspiration and bone core expressed high level of CD 73 (96.53%-97.89%), CD90 (98.19%-99.80%), and CD105 (85.02%-91.71%) (**Table 2**). Similar results were seen in the BMSCs from long bone and HGFs. However, the expression of another MSC marker, CD146, varied significantly among different samples. Long bone BMSCs exhibited the largest percentage (93.7%), followed by HGF (80.13%), osteotomy aspiration (75.02%), bone core (62.15%) and extraction aspiration (23.26%). The expression of CD45, a lymphocyte marker that was used as a negative selection marker, was very low in all of the cells (<6.12%).

Osteogenic differentiation potential

In the previously selected cells, osteogenic medium induced the cells to differentiate to osteoblast-like cells, which was characterized by increasing cellular ALP activities over time. HGFs were originally used as negative controls in this experiment. To our surprise, the HGFs that were used demonstrated a robust osteogenic potential demonstrated by the highest ALP activity in all samples (**Figure 5**). When focusing on the aBMSCs, cells from bone core had the highest ALP activity, followed by cells from extraction aspiration and osteotomy aspiration (**Figure 6**). Long bone BMSCs had a higher ALP activity than aBMSCs.

	CD73 (%)	***CD90 (%)***	***CD105 (%)***	***CD146 (%)***	***CD45 (%)***
BMSC *(iliac)*	99.96	99.43	99.19	93.70	0
Ext *(001)*	96.53	99.80	90.44	23.26	0.98
Imp-BM *(004)*	97.89	98.19	85.02	75.02	0
Imp-BC *(004)*	97.41	99.14	91.71	62.15	0.54
HGF	98.80	97.53	96.88	80.13	6.12

Table 2: Surface marker analysis using CD45, CD73, CD90, CD105 and CD146. BMSC represents human bone marrow stromal cells from iliac. Ext represents cells derived from extraction socket aspiration. 001 represents subject 001. Imp-BM represents bone marrow aspiration from implant osteotomy. Imp-BC represents bone core tissue from implant osteotomy. 004 represents subjects 004. HGF represents human gingival fibroblast.

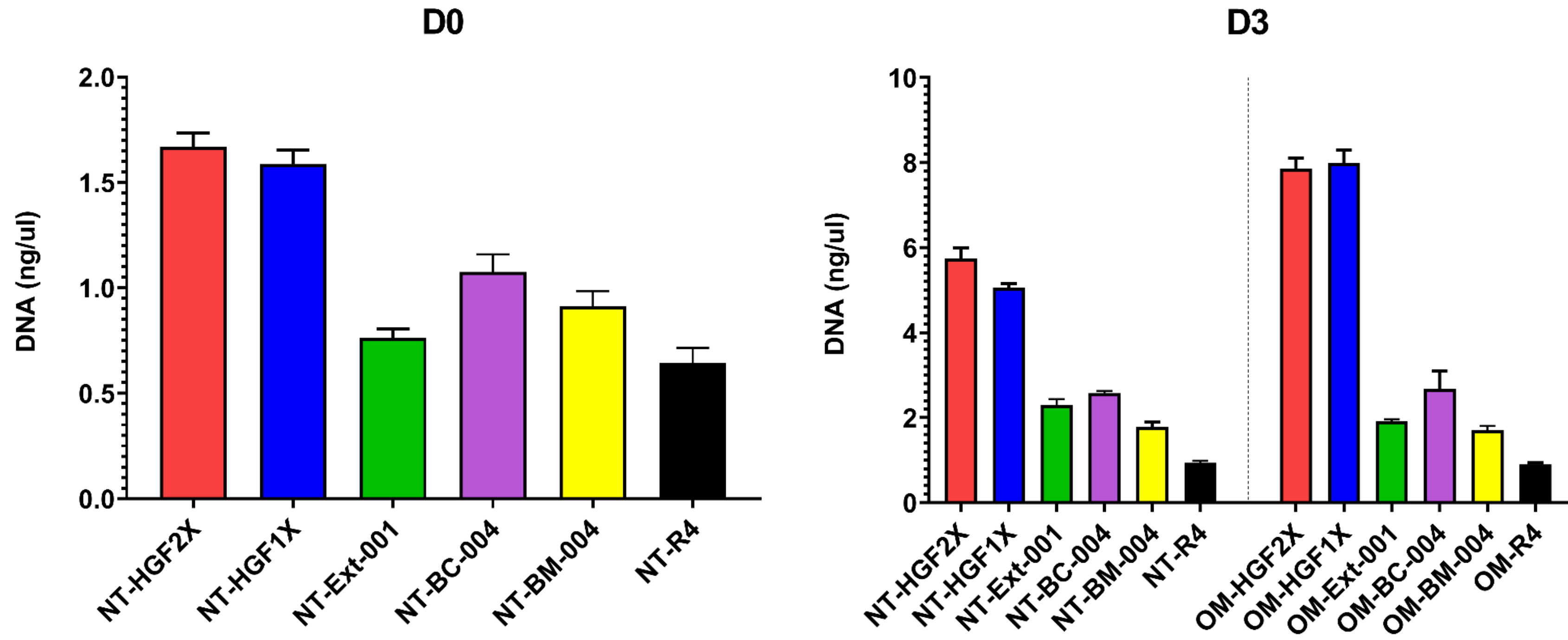


Figure 3: DNA contents on Day 0 and Day 3 of different samples. NT: no treatment. OM: osteogenic media. HGF2X represents human Gingival Fibroblast with 2X cell number. HGF1X represents human gingival fibroblast with 1X cell number. Ext-001: aspirate from extraction socket from subject 001. BC-004: bone core sample from subject 004. BM-004: bone marrow aspirate from osteotomy site from subject 004. R4: BMSCs from human iliac. DNA was measured in ng/ul. The mean of 3 or 4 replicates was presented

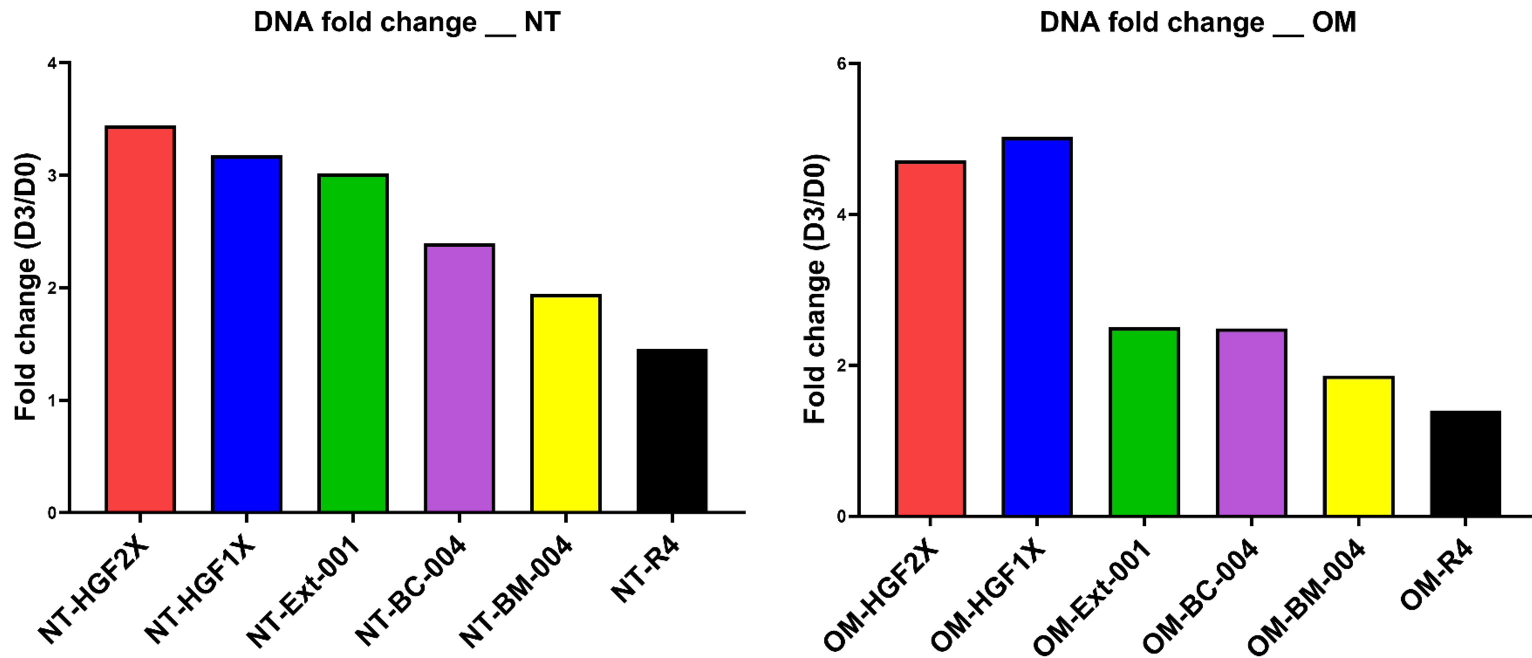
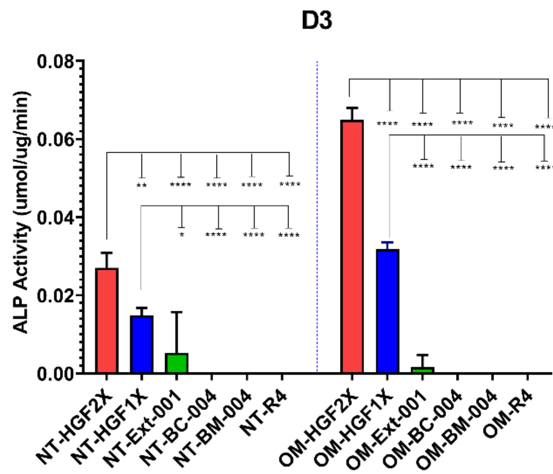
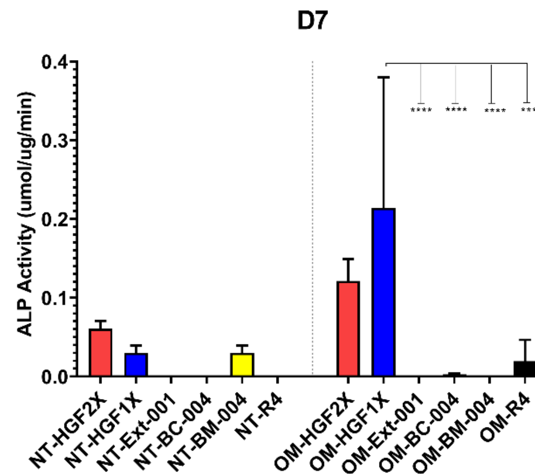
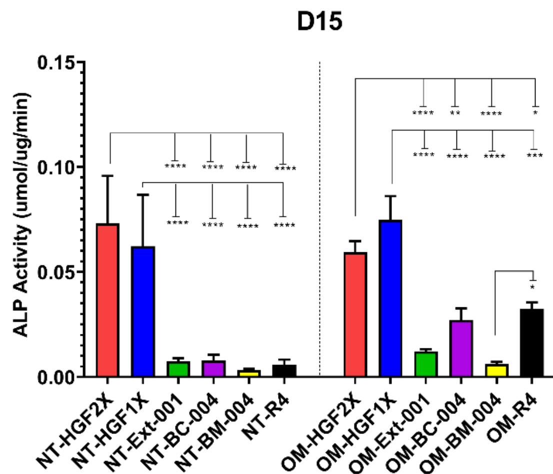


Figure 4: DNA fold changes. Rate of proliferation determined by the change of DNA content between Day 3 and Day 0, either under NT or OM treatment. NT represents the no treatment group. OM represents the osteogenic media group. HGF2X represents human Gingival Fibroblast with 2X cell number. HGF1X represents human gingival fibroblast with 1X cell number. Ext-001: aspirate from extraction socket from subject 001. BC-004: bone core sample from subject 004. BM-004: bone marrow aspirate from osteotomy site from subject 004. R4: BMSCs from human iliac.







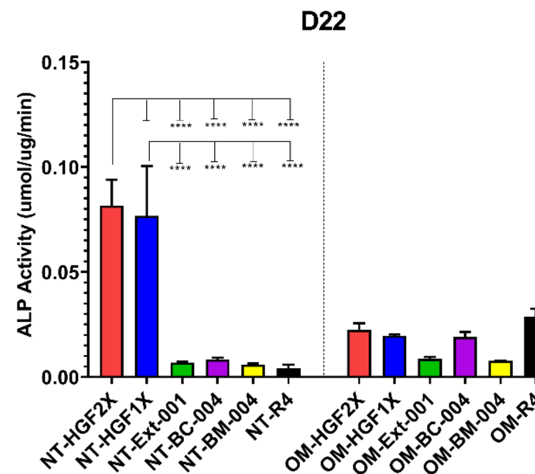


Figure 5: ALP activities of different samples after normalized to total protein content (umol/ug/min). NT: no treatment. OM: osteogenic media. Ext-001: aspirate from extraction socket from subject 001. BC-004: bone core sample from subject 004. BM-004: bone marrow aspirate from osteotomy site from subject 004. R4: BMSCs from human iliac. HGF1X represents human gingival fibroblast with 1X cell number, HGF2X represents human Gingival Fibroblast with 2X cell number. The mean of 3 or 4 replicates was presented. *: P < 0.05; **: P < 0.01; ***: P < 0.001; ****: P < 0.0001.

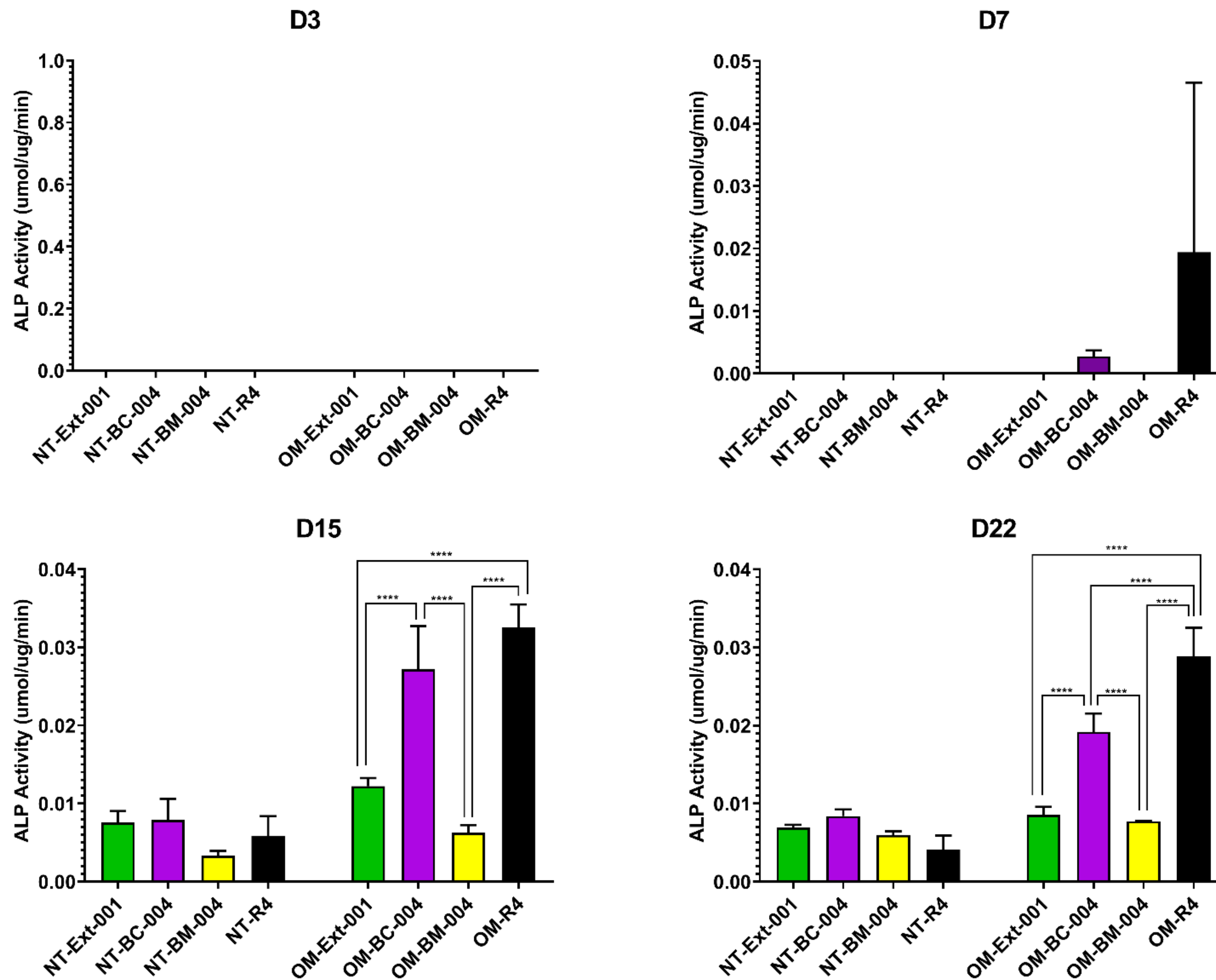


Figure 6: Comparison of ALP activities of MSCs from alveolar bone and long bone (umol/ug/min). Figure 4 was reformatted after the removal of HGFs. NT: no treatment. OM: osteogenic media. Ext-001: aspirate from extraction socket from subject 001. BC-004: bone core sample from subject 004. BM-004: bone marrow aspirate from osteotomy site from subject 004. R4: BMSCs from human iliac. The mean of 3 or 4 replicates was presented. ****: P < 0.0001.

Discussion

Historically MSCs haven been isolated from bone marrow of iliac crest[15]. Iliac crest harvest has been considered as the gold standard for obtaining MSCs, however, research has shown that the use of MSCs derived from different dental tissues may facilitate regeneration in animal models[36]. In this study different techniques were utilized to obtain MSCs through the use of bone core and marrow aspirate obtained during implant surgery as well as bone marrow aspirate obtained from extraction sockets. The results demonstrated different success rate of generating aBMSCs from different tissue sources, ranging from 40% to 80% with bone core samples being the most predictable sources in generating aBMSCs.

Matsubara et al.[31] also described the different success rates of generating aBMSCs from different techniques. They showed that extraction of wisdom teeth followed by marrow aspiration had greater success rates compared to dental implant aspiration after initial osteotomy. The authors suspected that this was related to the age of the subjects, as younger individuals were recruited for 3rd extraction compared to individuals recruited for implant surgery. The study did not examine bone core success rate at generating aBMSC. The results of the present study demonstrated that extraction aspiration success rates were slightly higher when compared to osteotomy aspiration, however, bone cores showed the highest success rates at generating aBMSC. This may be associated with the larger tissue volumes obtained from bone cores than those of marrow aspirates.

In a study by Mason et al[39] bone marrow samples were collected from 45 patients and aBMSCs were generated using three different techniques including; osteotomy aspiration, bone core, and bone core combined with osteotomy aspiration. The authors observed varying success rates with

the combination technique yielding a success rate of 100%, followed by the bone core technique at 97.5% and the osteotomy at 82% success rate. In comparison, success rates in the present study were lower with 40% for the osteotomy aspiration and 80% for the bone core technique. It is possible that the reduced success rates in the present study may be attributed to the small sample size of 12 patients.

When comparing population doubling times (PDT), the results of this study demonstrated that aBMSCs from extraction aspirates had higher proliferation rates compared to those of osteotomy aspirates and bone cores (43.62h vs 54.72h vs 49.46h). When comparing the osteotomy aspirate versus bone core techniques, Mason et al.[39] showed that proliferation was at least twice as fast for bone cores compared to osteotomy aspirates alone. These findings were supported by the present study in that bone core samples showed higher proliferation rates compared to the osteotomy aspirates however the rate was not twice as fast.

MSC characterization was accomplished by studying different cell surface markers by using fluorescence-activating cell sorting in the three types of samples. All of the aBMSCs from extraction aspiration, osteotomy aspiration and bone core expressed high levels of CD 73 (>96%), CD90 (> 98%), and CD105 (>85%). These results were also seen in long bone and HGFs. Mason et al.[39] evaluated aBMSCs characteristics and found high levels of CD73, CD 90 and CD105 in their samples. Matsubara et al.[31] examined the difference between cell surface antigens of alveolar and iliac bone marrow stromal cells and showed that none of the cell surface antigens differed between the two groups. This was also the case in the current study with the exception that a large variance was observed in regard to CD146, in which iliac BMSCs showed higher expression than aBMSCs.

When comparing osteogenic potential Matsubara et al.[31] showed that iliac BMSCs had similar ALP activity when compared to aBMSCs. The results of the current study suggest that ALP activity in iliac BMSCs was higher than that in cells from bone core, osteotomy aspiration, and extraction aspiration. Although this study used a small sample size, the results indicated that MSCs derived from jaw bones and long bones may have different properties. Originally, HGFs served as a negative control in the study. In unexpected results, the HGFs tested in this study demonstrated a robust proliferation potential and a much stronger ALP activity than each of the other cells. This difference may be attributable to the genetic background of this specific donor. Another possible explanation is that MSCs can also be found in gingival tissues[41]. In the future, it may be interesting to study the regenerative potential of HGFs.

Several limitations were noted within this study. The sample size consisted of only 12 subjects, which is not an adequate size to achieve any statistical relevance. Although the study obtained samples in a standardized method, errors may possibly have occurred during collection, transportation, and/or analysis of the samples. Due to the small sample size it was not possible to evaluate the effects of sex, age, and health status on generating aBMSCs from different tissue sources. In addition, there was no attempt to account for differences in bone density among samples, grafted versus non grafted sites, maxilla versus mandible, and bone core volume. Another limitation was that in vivo bone formation of the MSCs obtained from the patients was not tested. It is also important to note that MSC can be obtained from PDL cells from during extraction of 3rd molars[35,42,43] and from the dental pulp[33]. Finally, this study did not compare the differences between aBMSCs with MSCs from other dental tissues.

Overall, this study compared three different techniques to obtain aMBSCs: extraction socket aspiration, osteotomy aspiration and bone core digestion. Further research is required to evaluate

the potential use of aBMSCs in periodontal regeneration and other regenerative procedures in dentistry.

Conclusion

This study compared three different methods to obtain aBMSCs, including: extraction socket aspiration, osteotomy aspiration and bone core digestion. Bone core samples obtained during implant surgery may be a more reliable source for generating aBMSCs than extraction socket aspiration and osteotomy aspiration. MSCs derived from different methods may have different characteristics in regard to proliferation, differentiation potential and cell surface markers.

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