

P54: Nonviral-Mediated Gene Therapy of Adipose-Derived Stem Cells

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INTRODUCTION: Tissue regeneration strategies have included the delivery of recombinant proteins, viral-mediated and nonviral-mediated gene therapies. The high dosing requirements, expense, and safety issues have limited the potential of recombinant protein delivery. Safety issues continue to be a concern for viral-mediated gene therapy. Nonviral-mediated gene therapy continues to be limited by low transfection efficiencies. Enhancing nonviral-mediated gene therapy by improving uptake and expression is clinically attractive. For the present studies, several types of plasmid DNA-polymer complexes were evaluated *in vitro* for their ability to transfect and express enhanced green fluorescent protein (eGFP) in adipose-derived stem cells (ADSCs).

METHODS: The eGFP was spliced into a plasmid vector utilizing the elongation factor 1 alpha promoter with an enhanced CMV element (pCEF1a-DNT-eGFP) and purified by Mobius 1000 Plasmid Kits (Novagen, Madison, WI). Following the initial isolation procedure, ADSCs were plated at a seeding density of 25,000/well in 12-well plates (n=3). ADSC purity was evaluated with the putative ADSC marker CD49d at passage 2 and 3. Five plasmid DNA-polymer complexes, as well as controls, were exposed to the ADSCs for 24 hours. After a 3 day exposure period, the cells were removed by trypsin, washed and scanned for transfection efficiencies by a fluorescence-activated cell sorter (FACStar cell sorter with FACScan instrument, Becton-Dickinson). Cells expressing GFP are presented as a percentage of ADSCs expressing GFP vs. total cell number. Single-factor ANOVA with post-hoc analysis was used to determine statistical significance with $p < 0.05$ established as significant.

RESULTS: Initial characterization at P2 and P3 with the putative ADSC marker CD49d has exhibited purities of 73% and 68%, respectively, as determined by FACS analyses. The percentages of ADSCs expressing GFP at 3 days following the transfection procedures are illustrated in Figure 1. Overall, the variable configurations influenced GFP expression ($p < 0.001$). The ADSCs exposed to the DNA:TROjane group expressed statistically more GFP than the other groups ($p < 0.05$), with the exception of jetPEI. The FuGene reagent exhibited the lowest toxicity, while the liposomes and CaPO₄ groups were consistently toxic.

CONCLUSION: The DNA:TROjane complex was the most effective nonviral-mediated vector complex for transfection of ADSCs in the present studies. The expression cassette from the present studies has been modified to contain and express VEGF and IGF-1. DNA:TROjane complexes with plasmid DNA expressing VEGF and IGF-1 are being evaluated to improve tissue-engineered implant vascularity and ADSC durability, respectively.

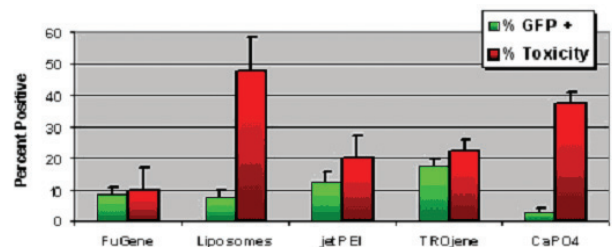


Figure 1. Percentage of GFP expression and toxicity

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P55: Novel Approach to Closed Treatment of Prominent Ear without Skin Resection (Endotoplasty)

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INTRODUCTION: The aim of this study is to demonstrate an alternative closed approach to treat prominent ear, without skin resection, performed through a single 5-8mm incision located on the external border of the helix at the exit of the posterior crus. The strategy is delineated by anterior and posterior global undermining of the auricular skin over the anti-helix and the concha; controlled anterior anti-helix rasping with stabilization of the antihelical row using trans-cutaneous stitches, and resection of the hypertrophic concha according the “conchal show” principles.¹ Considering the minimum access, preservation of the auricular structure and the closed modelation of the auricular cartilage, the term Endotoplasty is proposed for this procedure. (Fig. 1)

PATIENTS AND METHODS: The surgery was performed on 342 patients (214 female and 128 males, mean age 22.7 years), and included 328 bilateral and 14 unilateral cases(n=670 ears), over a period of five years. The data was classified according to the gradation proposed by the Egloff et al.² Based on this classification, 178 patients belonged to the type I(altered anti-helix associated with conchal hypertrophy), 6 patients to the group II(hypertrophied concha and normal anti-helix), 132 to the group III(altered anti-helix, normal concha), and another 26 to the group IV (association with one of the former variation with a lateral lobe).

RESULTS: A balanced external ear configuration was achieved in majority of the cases.³ A smooth antihelix surface was consistently achieved with few complications. The most frequent complication was sinus formation around the knots of the trans-cutaneous stitches and occurred in 12 patients(3,5%). This inconvenience reduced dramatically, when the stitches were done with either unabsorbable 5-0 clear nylon (Ultralon®-Biosut-MG) or absorbable 5-0 PDS(Sommerville-Eticon®). Moderate asymmetry were present in 5 patients (1,4%). It was correct utilizing the same strategy. Hematoma occurred in 3 patients(0,8%). No infection or pathologic scar were registred. The mean follow-up period ranged from six months to five years.(Fig. 2).

In order to measure the patient satisfaction, the Caouet-Laberge et al.⁴ questionnaire was applied. 98% of the patients scored to be very satisfied or satisfied.

DISCUSSION: In this investigation, a closed method to correct the prominent ear is evaluated. The entire procedure can be performed through a single 5-8mm incision located on the external border of the helix and the exit of the posterior crus. The strategy starts with mandatory tumescent infiltration, to facilitate the undermining over the anti-helix and the concha, both in front and behind the ear. The cartilaginous mold stays free to be sculptured. The global shrinkage of the skin envelope contributes to the medianization of the auricle, making skin resection unnecessary.⁵ The instrumentation is composed of a skin descolorator, one direction thin rasp, (similar to the one utilized to undermining and smooth the nasal dorsum) and one full curved 15cm Fomon pair of scissors . Eletrocautery is not utilized. The surgeon can accomplish the whole treatment without auxiliar.

Although not within the scope of this study, histological analysis before and after controlled sequence of rasping, demonstrated that only 30 to 40% of the auricular thickness is removed, and this promotes the best contra-lateral curvature according Stenström.^{6,7} The risk to transpose the cartilaginous layers causing irregular contouring is minimal, and it was not observed in any of our 342 patients (n= 670 ears).

The hypertrophic concha present in the types I, II and IV had to be corrected in more than 60% of the auricles. To address this problem, we embodied the concept of “conchal show” advocated by Vermellian and cols. They analyzed 100 patients with clinically normal ear, recording the ideal conchal width, which varied from 8 to 11mm. We considered conchal width of 10mm as the limit. The excess is resected in order to promote medianization of the auricula. (Fig.3)

CONCLUSION:The closed approach, performed through a single small incision on the external border of the helix, at the union of the exit of the posterior crus, can correct effectively prominent ear types I, II and III of the Egloff grading. This strategy requires tumescent infiltration with anterior and posterior undermining of the auricula; controlled anterior scratching of the anti-helix with its stabilization using trans-cutaneous stitches and correction of the hypertrophic concha according the “conchal show” principles.

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FIGURES

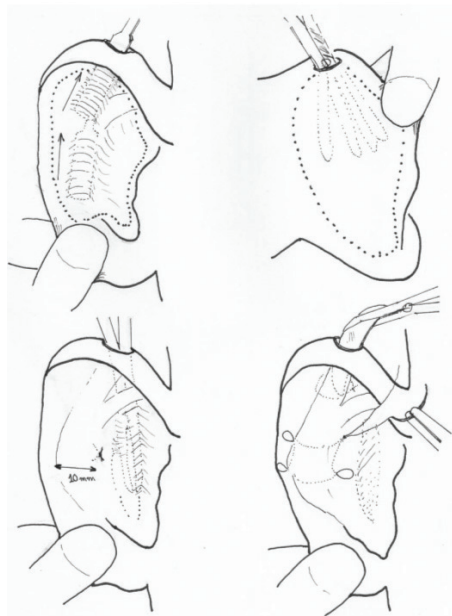


Figure 1. (Above, left) Schematic drawing showing the small surgical access utilized in this study to perform the closed strategy. The dotted lines demonstrate the extension of the anterior skin undermining. The arrows give the direction of the anterior rasp in order to achieve “C” shape resultant anti-helix - cranial strokes, starting from the lower part to the middle point of the anti-helix and from this site to the superior crura. (Above, right) Posterior undermining (dotted lines) using a Metzembaun scissors, leaves the conchal cartilage free to be trimmed; (Below, left) The correct amount of the hypertrophic concha is designed over the medial skin. A full curved 15mm Fomon pair of scissors is introduced, runs behind of the con-

chal cartilage and breaks the cartilage just under the inferior crura, connecting the anterior and posterior conchal spaces. The “conchal show” is observed, leaving 1 cm from the external border of the anti-helix roll to the concha. At this level a 5-0 nylon suture keep temporarily the detached skin again to the cartilage in order to prevent overresection. The convex side of the scissors faces the auditory canal and bites the medial part of the concha. The resection is completed with the convex side of the scissors facing the external border, at the 1cm limit. (Below, right) The semilunar cartilaginous piece is produced through the same incision with a kelly forceps. The rasp smoothed the remaining step closed the auditory canal. Finally, two transcutaneous stitches with the knots buried anteriorly at the triangularis fossa and under the inferior crura, stabilizes the anti-helical row.



Figure 2. A one year post-operative view of a 7 year-old young girl with ethnic skin, with prominent ear grade I. The back view shows the good skin retraction, good ear medianization, without scar.



Figure 3. The small access utilized to the closed approach located on the external border of the helix at the junction of the superior crus of the anti-helix.

Posters

P56: Ocular Complications Following Bilateral Eyebrow Laser Photothermolysis

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INTRODUCTION: Iris atrophy and pupillary irregularity following bilateral eyebrow laser photothermolysis.

METHODS: A 39 year old caucasian female with a past ocular history of bilateral LASIK eye surgery underwent laser photothermolysis of the eyebrows and lower legs. The laser used was a long-pulsed 755nm wavelength infrared alexandrite (Cynosure, Inc., Chelmsford, MA, USA). Pulse duration was 20 msec with a spot size of 12.5 mm. Initially, safety glasses were used during laser epilation of the legs. The safety glasses were subsequently removed during laser epilation of both eyebrows. The patient reports the technician used his left hand to shield her right eye while treating her right eyebrow, and shielded both eyes with his hand when treating the left eyebrow. The eyebrows were treated both above and below the natural eyebrow line.

RESULTS: Six hours later, the patient noticed blurred vision, sensitivity to lights and pain in her left eye (OS). She presented to an ophthalmologist approximately 24 hours after the procedure. She was diagnosed with non-granulomatous iritis OS and placed on topical prednisolone. Initial vision was 20/20 in both eyes with no evidence of cataract or other ocular abnormalities. On exam 4 days later, the patient had pigmented cells in the anterior chamber and 4-5 clock hours of iris transillumination defects with iris atrophy infero-temporally. The pupil was distorted supero-temporally and 5 clock hours of posterior synechiae between the iris and anterior lens were seen temporally. She was subsequently placed on Atropine 1% in an attempt to break the synechiae and relieve the pupillary distortion. Over 8 weeks, the iritis resolved and her vision remained 20/20. The posterior synechiae remain, but have since decreased to 1-2 clock hours after using topical tropicamide for 4 weeks. Iris transillumination defects and pupil distortion remain. There is no cataract formation to date.

CONCLUSIONS: Laser hair removal has become increasingly popular, and in 2004 it was the second most requested non-surgical cosmetic procedure. The most common lasers for hair removal include the Nd:YAG (wavelength of 1064 nm), alexandrite (755nm) and diode (810nm). Cases of ocular injury after eyebrow epilation including pupil irregularities and cataract formation have been reported after using the diode laser, but this is the first case reported after using the infrared alexandrite.

Most lasers use selective photothermolysis by targeting melanin in the dermis hair follicle as the chromophore. The alexandrite laser can possibly penetrate the thin skin of the eyelid and damage ocular structures containing melanin (including the iris). The Bell's response when an eyelid is closed could place the iris closer to the laser source, thereby increasing the chance of damage. This case demonstrates a possible risk to ocular tissues while performing laser hair epilation. We feel that patients should be made aware of the possibility for ocular damage when considering laser eyebrow epilation. In addition, if the patient wishes to pursue the treatment, ocular shields should be worn throughout the entire procedure.

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P57: Oromandibular Reconstruction Using Radial Forearm Osteocutaneous Flap with Calcium Phosphate Cement (CPC)

Mitsuo Hatoko, MD

INTRODUCTION: The radial forearm flap is one of the most frequently used skin flaps in reconstruction of oral cavity defect. In surgery for oral cancer, resection is often performed not only the oral mucosa but also the mandibular bone, resulting in an oromandibular defect. The radial forearm flap can be utilized with radial bone, but the amount, which can be harvested without bone fracture, is limited. Therefore, when the defect of the mandibular bone is large, another kind of vascularized bone, non-vascularized bone, or artificial material must be considered. However, forearm skin flap is often the best material to cover an oral skin defect because it has the characteristics which is desirable to reconstruct oral skin and mandibular bone defect: thin skin, a long vascular pedicle.

MATERIALS: The calcium phosphate cement (CPC)(Biopex[®], Mitsubishi Material Corporation, Tokyo, Japan) used in this study was developed in JAPAN.

CASE REPORT: A 61 year-old female had suffered a left gingival tumor. The tumor was resected with a part of the mandibular bone. The defect was 15mm (bottom)×50mm (length).

A free radial forearm osteocutaneous flap was harvested with a bone segment measured 10mm in width and 50mm in length. After anastomosing radial artery and its comittant vein with superior thyroid artery and vein, respectively, the harvested radial bone was fixed with titanium microplates to create smooth alveolar ridge and the space between the residual mandibular and grafted radial bone was filled with 2cc of CPC. The radial bone and CPC was covered with flap skin.

The radial forearm osteocutaneous flap was viable. The postoperative X-P findings revealed that a good contour to the alveolar ridge and the CPC-injected space was filled with a uniform, high-density mass without significant volume reduction of the injected cement.

The patient was able to wear a partial dental prosthesis successfully. Concerning flap donor site, movement of hand was also with no problem.

CONCLUSION: The combined application of radial forearm osteocutaneous flap with CPC is a useful reconstructive option in the selective case with olomandibular defect.



Figure 1. After resecting the tumor.

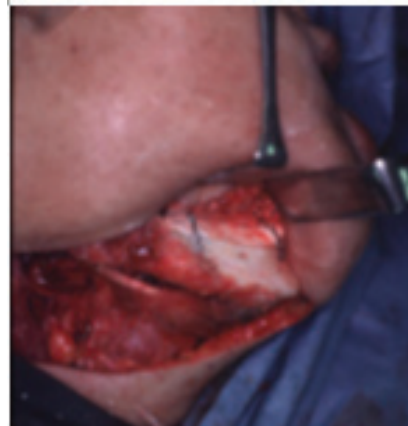


Figure 2. The radial bone was fixed, and injected calcium phosphate cement to fill the apace.



Figure 3. Twelve months after the operation.

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P58: One Stage Integra® and Skin Grafting of Full-Thickness Human Wounds

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PURPOSE: The synthetic dermal regeneration template Integra® (Integra Life Sciences, Inc., Plainsboro, N.J.) is currently used in a two-stage procedure that allows vascularization of the matrix prior to placing a skin graft. If this could be performed as a one-stage procedure, it would result in less cost and morbidity to the patient. Using sub-atmospheric pressure treatment (V.A.C., K.C.I., Inc., San Antonio, TX) as a dressing for Integra has previously allowed one-stage engraftment of Integra in an animal model. We now report our initial experience with one-stage Integra use in a clinical series.

METHODS: Patients were chosen for this technique when skin graft alone was not deemed suitable as a reconstructive option. Each patient underwent the usual surgical wound care used for a two-stage approach. The Integra was meshed 1:1 and the silicone layer was separated from the dermal matrix portion. The dermal matrix was placed in the wound and covered with a thin split-thickness skin graft and a non-adherent dressing. A subatmospheric pressure dressing was then applied and left in place for a period of at least 7 days.

RESULTS: Fifteen patients (Age 36-76) were evaluated. The wound size was 4 – 250 cm² (mean = 79 cm²). Etiology of wound was release of burn scar contractures in two, chemical injury in one, trauma defects in 4, and cancer resection in one. Exposed in the wound was tendon (n= 4), bone (n = 4), open fracture (n = 2) and two with soft tissue alone. Healing was complete in 7 cases without the need for re-operation. In one, the Integra and skin graft was lost associated with an interruption of the subatmospheric pressure dressing.

CONCLUSIONS: Using subatmospheric pressure dressings, Integra may be successfully used for one-stage coverage for burn reconstruction and other challenging wounds.

P59: One-Stage Abdominal Wall Herniorrhaphy and Body Contouring in the Morbidly Obese Patient

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PURPOSE: Incisional hernias in the abdominal wall are a frequent and serious problem in the post-operative morbidly obese patient, particularly in the post bariatric surgery patient. Diagnosing and addressing abdominal wall hernias is a critical step in the preoperative planning of any abdominal body contouring procedure. Because of the high risk of surgical complications and morbidity in this population, a single-stage procedure simultaneously addressing hernia repair and lipectomy would offer a significant contribution to the management of these patients.

METHODS: We performed a retrospective review of all consecutive one-stage hernia repair and body contouring procedures performed by the senior authors from 1999-2005. Patient demographics, operative techniques, and perioperative parameters were reviewed. After accessing the abdominal wall through a direct lipectomy incision, tension free hernia repair was achieved in all patients with external oblique fascial release and underlay tissue separating mesh when necessary. Postoperative complication rates and hernia recurrence rate were used as endpoints to assess the safety and efficacy of the procedure.

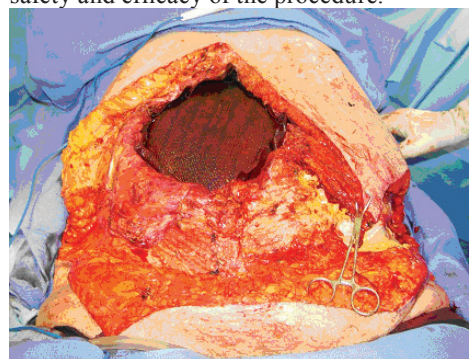


Figure 1.

RESULTS: 48 patients, 8 males and 40 females, with a mean BMI of 38 are presented. Mean follow-up after surgery was 203 days. 20 patients (42%) had prior history of bariatric surgery. 20 patients (42%) had a primary hernia and 28 patients (58%) presented with recurrent hernia after previous failed repair. Overall complication rate was 24%. Complications included wound infection, hematoma, seroma, and partial flap loss. Hernia recurrence rate was 4%, with both hernias occurring at sites distant from the original repair.

CONCLUSIONS: 1. Identification of all abdominal wall defects, and tension free repair with adjunctive fascial release and underlay, tissue separating mesh when necessary results in low hernia recurrence rates. 2. Direct lipectomy incisions provide wide exposure to the abdominal wall and offer excellent access for hernia repair. 3. Herniorrhaphy and body contouring procedures can be safely combined to offer a one-stage procedure with low perioperative morbidity, low hernia recurrence rate, complication rate comparable to that seen with body contouring procedures alone.