Cranioplasty with Adipose-Derived Stem Cells, Beta-Tricalcium Phosphate Granules and Supporting Mesh: Six-Year Clinical Follow-Up Results

TUOMO THESLEFF [ID],[a] KAI LEHTIMÄKI,[a] TERO NISKAKANGAS,[a] SANNA HUOVINEN,[b]
BETTINA MANNERSTRÖM,[c] SUSANNA MIETTINEN,[d] RIITTA SEPPÄNEN-KAIJANSINKKO,[c] JUHA ÖHMAN[a]

Key Words. Adipose stem cells • Tissue engineering • Biomaterials • Beta-tricalcium phosphate • Cranial repair • Outcome

[a]Department of Neuroscience and Rehabilitation, Tampere University Hospital, Tampere, Finland; [b]Department of Pathology, Fimlab Laboratories, Tampere University Hospital, Finland; [c]Department of Oral and Maxillofacial Diseases, University of Helsinki and Helsinki University Hospital, Finland; [d]Adult Stem Cells, BioMediTech, Faculty of Medicine and Life Sciences, University of Tampere, Finland Science Center, Tampere University Hospital, Finland

Correspondence: Tuomo Thesleff, M.D., Department of Neuroscience and Rehabilitation, Tampere University Hospital, P.O. Box 2000, FI-33521 Tampere, Finland. Telephone: +358-3-311-63023; e-mail: tuomo.thesleff@pshp.fi

ABSTRACT

Several alternative techniques exist to reconstruct skull defects. The complication rate of the cranioplasty procedure is high and the search for optimal materials and techniques continues. To report long-term results of patients who have received a cranioplasty using autologous adipose-derived stem cells (ASCs) seeded on beta-tricalcium phosphate (betaTCP) granules. Between 10/2008 and 3/2010, five cranioplasties were performed (four females, one male; average age 62.0 years) using ASCs, betaTCP granules and titanium or resorbable meshes. The average defect size was 8.1×6.7 cm^2. Patients were followed both clinically and radiologically. The initial results were promising, with no serious complications. Nevertheless, in the long-term follow-up, three of the five patients were re-operated due to graft related problems. Two patients showed marked resorption of the graft, which led to revision surgery. One patient developed a late infection (7.3 years post-operative) that required revision surgery and removal of the graft. One patient had a successfully ossified graft, but was re-operated due to recurrence of the meningioma 2.2 years post-operatively. One patient had an uneventful clinical follow-up, and the cosmetic result is satisfactory, even though skull x-rays show hypodensity in the borders of the graft. Albeit no serious adverse events occurred, the 6-year follow-up results of the five cases are unsatisfactory. The clinical results are not superior to results achieved by conventional cranial repair methods. The use of stem cells in combination with betaTCP granules and supporting meshes in cranial defect reconstruction need to be studied further before continuing with clinical trials. STEM CELLS TRANSLATIONAL MEDICINE 2017;00:000–000

SIGNIFICANCE STATEMENT

In this article we report long-term results of five patients who received a cranioplasty using autologous adipose-derived stem cells seeded on beta-tricalcium phosphate granules. The initial results were promising, with no serious complications. Nevertheless, the 6-year follow-up results of the five cases are unsatisfactory. The use of this strategy for cranial defect reconstruction must be studied further before continuing with clinical trials and before applying the method in clinical practice.

INTRODUCTION

Skull restoration remains a challenge for neurosurgeons and plastic surgeons [1]. The number of patients in need of cranioplasty is increasing because of continuous improvements in neurosurgical critical care and a growing number of decompressive craniectomies performed [1]. Common complications in cranial repair surgery include for example infection, wound dehiscence, intracranial hemorrhage, resorption, and/or dislocation of the graft [2–5]. The reported complication rate of cranioplasties today is 16%–40%, with a general reoperation rate of 25% [6–10]. Patients' autologous removed and stored bone has been considered as the gold standard in cranial vault reconstruction, but there are studies reporting superior results achieved with synthetic materials such as hydroxyapatite, bioactive fiber-reinforced composite, polymethylmethacrylate, or titanium [9, 11, 12].

Our aim was to overcome common problems associated with conventional cranioplasties by creating bone from patients' own adipose-derived stem cells (ASCs), beta-tricalcium phosphate (betaTCP) granules and supporting meshes [13, 14]. In recent years, ASCs have been under extensive study in regenerative medicine [15–18]. They are easily retrieved in high numbers from fat

Table 1. Characteristics of patients and procedures

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	F	M	F	F	F
Age at primary cranioplasty	60	59	62	75	54
Diagnosis	Skull hemangioma	Frontal meningioma	Acute SDH	Cranial base meningioma	Skull meningioma
Indication for cranioplasty	Primary operation	Loosening of the acryl graft	Bone flap infection	Bone flap infection	Primary operation
Defect size (length × height, cm)	6.9 × 6.4	9.0 × 7.5	8.9 × 6.8	6.5 × 3.7	9.0 × 9.3
Inner mesh	No	No	Resorbable	Resorbable	Titanium
Outer mesh	Titanium	Resorbable	Resorbable	Resorbable	Resorbable
Total number ASCs implanted (ASCs per ml)	4,140,000 (37,637)	8,712,000 (108,900)	3,982,500 (49,781)	7,821,000 (391,050)	2,812,500 (28,125)
Indication for re-operation	Late infection	Partial resorption and loosening of the graft	—	Total resorption	Recurrence of the meningioma in the dura
Time between cranioplasty and re-operation (years)	7.3	0.9	—	2.0	2.2
Re-operation technique	Revision, removal of the graft	Titanium mesh applied on the defect	—	Titanium cranioplasty	Re-craniotomy and excision of the dural meningioma
Histological analysis of the graft	Osteonecrosis and acute to subacute osteomyelitis	Partly necrotic islets containing loose collagen and poorly maintained osteoblasts. Focally some well-formed bone trabeculae containing vital osteocytes	—	—	—
Notice	Frontal sinus opened in the primary operation and filled with bone cement	Remaining graft was hard and fixated with screws into a titanium mesh.	Resorption of the graft in the skull x-ray	Abdominal hematoma at the adipose tissue harvest site	Graft was successfully ossified and had grown into the surrounding bone
Clinical follow-up (years)	7.4	6.8	6.2	6.5	6.1

Abbreviations: —, no data; ASC, adipose-derived stem cell.

tissue and expanded in vitro, which makes them an attractive tool in tissue reconstruction [14, 19, 20]. However, scientific evidence in terms of safety and efficacy of ASC use in regenerative medicine is still limited [14, 20–24].

We performed five cranioplastic surgeries using ASCs seeded in betaTCP granules supported by resorbable or titanium meshes to patients who had moderate-sized cranial defects of varying etiologies. The grafts seemed to be successful in short-term follow-up, both clinically and radiologically, but already the mid-term results showed increased graft resorption [13, 14]. In the current study, our objective is to report the long-term results (6.1–7.4 years) of this method.

MATERIALS AND METHODS

Five patients, four females and one male, average age 62.0 years (range, 54–75 years), underwent cranioplasty using this novel technique between 10/2008 and 3/2010. The average defect size was 8.1×6.7 cm^2 (range, $6.5 \times 3.7 - 9.0 \times 9.3$; Table 1). Primary diagnoses and indications for cranioplasties are presented in Table 1.

Adipose tissue harvests from subcutaneous abdominal fat (approx. 100 ml) were performed approximately 3 weeks prior to the cranioplasty procedure. The fat together with 60 ml of autologous serum was transported to Regea Institute for regenerative medicine, University of Tampere for stem-cell isolation and expansion. None of the cranioplasty constructs was supplemented with rhBMP-2.

ASC Isolation and Preparation for Transplantation

ASCs were isolated and expanded in vitro in clean rooms according to standard operating procedures and following Good Manufacturing Practice guidelines at Regea. Details of the isolation and expansion procedures with minor modifications are described in a previous publication [20]. Recombinant collagenase NB 6 (Invitrogen, Paisley, Scotland, United Kingdom; Good Manufacturing Practice grade; SERVA Electrophoresis GmbH, Heidelberg, Germany) was utilized to mince and digest the adipose tissue. The isolated ASCs were expanded for approximately 22 days in basal media containing Dulbecco's modified Eagle medium/F-12 (Gibco Invitrogen, Paisley, Scotland, United Kingdom) with 15% of autologous serum, without antibiotics. Subsequently the cells were passaged on confluence and detached mechanically with a cell scraper and prepared for cell transplantation. To allow cell attachment, approximately 15×10^6 cells of passages 3 to 4 were combined with 60 ml of betaTCP granules (Chronos1, porosity 60%, granule size 1.4–2.8 mm; Synthes, Oberdorf, Switzerland) 48 hours before the operation. The cells were tested negative for Mycoplasma contamination as determined by a Mycoplasma PCR kit (VenorGem; Minerva Biolabs GmbH, Berlin, Germany) and cell sterility and
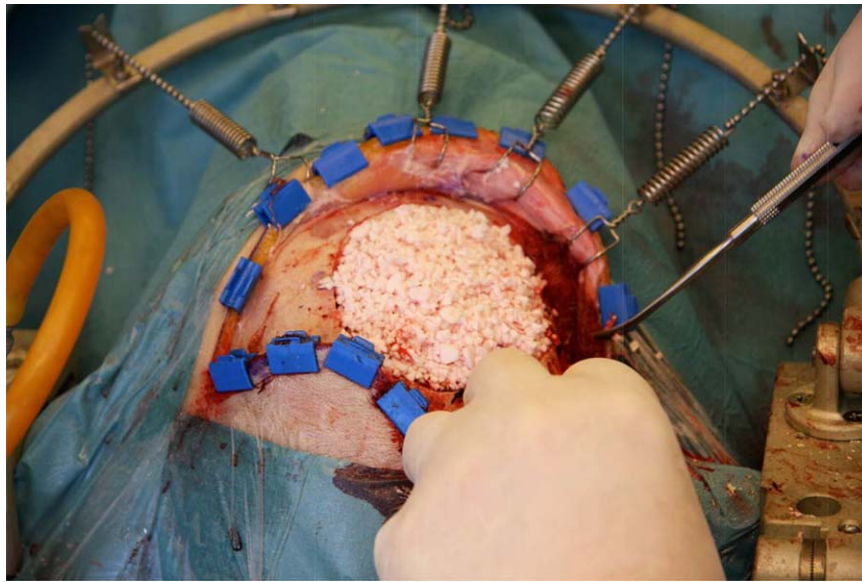
Figure 1. Intraoperative photograph of a meningioma resection site filled with autologous adipose stem cell-seeded beta-tricalcium phosphate (β-TCP) granules (Patient 5).

endotoxins were tested by Biovian Ltd. (Turku, Finland) according to methods described in the European Pharmacopoeia (Council of Europe, Strasbourg, France) [25].

In Vitro Analyses and Live/Dead Staining

For the in vitro analyses, ASCs from all patients were expanded in basal media, except in the osteogenic differentiation capacity assessment, in which commercially available human serum (Bio-Whittaker; Lonza, Walkersville, Maryland), was used because of the limited amount of autologous serum.

Cell attachment to the betaTCP granules and the cell viability were studied using Live/Dead staining before the operation. Briefly, the cell-biomaterial combination was incubated with a mixture of CellTracker green (5-chloromethylfluorescein diacetate) and ethidium homodimer (Molecular Probes, Eugene, Oregon). The viable cells (green fluorescence) and dead cells (red fluorescence) were detected with a fluorescence microscope.

Flow Cytometric Surface Marker Expression Analysis

ASCs were further expanded in vitro and were analyzed at passages 4 to 5 by flow cytometry (FACSAria; BD Biosciences, Erembodegem, Belgium). Monoclonal antibodies against CD9-PE, CD10-PECy7, CD13-PE, CD14-PECy, CD19-PECy7, CD29-APC, CD49d-PE, CD73-PE, CD90-APC, CD106-PE-Cy5, CD146-PE, and CD166-PE (BD Biosciences); CD45-FITC (Miltenyi Biotech, Bergisch Gladbach, Germany); CD31-FITC, CD34-APC, CD44-FITC, HLA-ABC-PE, and HLA-DR-PE (Immunotools GmbH, Friesoythe, Germany); and CD105-PE (R&D Systems Inc., Minneapolis, Minnesota) were used. Analysis was performed on 10,000 cells per sample. The positive expression was defined as the level of fluorescence greater than 99% of the corresponding unstained cell sample.

Osteogenic Differentiation Capacity Assessment

For the in vitro osteogenic differentiation analyses, surplus cells were maintained in osteogenic media containing basal media supplemented with 15% human serum, 50 μM L-ascorbic acid 2-phosphate (Sigma-Aldrich, St. Louis, Missouri), 10 mM beta-glycerophosphate (Sigma-Aldrich), and 100 nM dexamethasone (Sigma-Aldrich). Control cell cultures were maintained in basal media supplemented with human serum. The cells were seeded at a density of 2.5×10^3 cells/cm^2 and cultures were subsequently analyzed by alkaline phosphatase staining at day 14.

Cranioplasty Procedure

All patients received routine preoperative cefuroxime antibiotic 1.5 g intravenously (Zinacef, GlaxoSmithKline, United Kingdom). At first the dura was exposed in all cases. In Patient 1, a hemangioma was removed with the outer and inner diploe of bone, and the opened frontal sinus was cranialized and filled with bone cement (NORIAN Reinforced Fast Set Putty, Synthes, Oberdorf, Switzerland, http://www.synthes.com). In Patient 2, a loosened acryl graft was removed. In Patients 3 and 4, the skin was carefully dissected loose from the dura. In Patient 5, a bone meningioma was resected. Next, ASCs combined with betaTCP granules were applied in the defect (Fig. 1). The numbers of implanted ASCs are presented in Table 1. To keep the betaTCP granules in place, resorbable custom-molded mesh (PLGA, 85:15 poly (L-lactide-co-glycolide)) (RapidSorb, Synthes, Oberdorf, Switzerland, http://www.synthes.com) or titanium mesh was used either in single or bilaminate fashion (Table 1). Meshes were secured with either titanium or resorbable screws.

Follow-Up

Patients were followed at 1 week, 1, 6, and 12 months after surgery and annually thereafter. During the first year, Houndsfield units (HU) were measured from CT studies to assess bone density. After the first year, imaging studies were done based on clinical consideration, and HU measurement was not systematically continued. Histological samples of the graft were obtained from two re-operated patients (Patients 1 and 2). The formalin-fixed, paraffin-embedded sample sections were stained with hematoxylin and eosin and periodic acid-Schiff, and histologically evaluated.
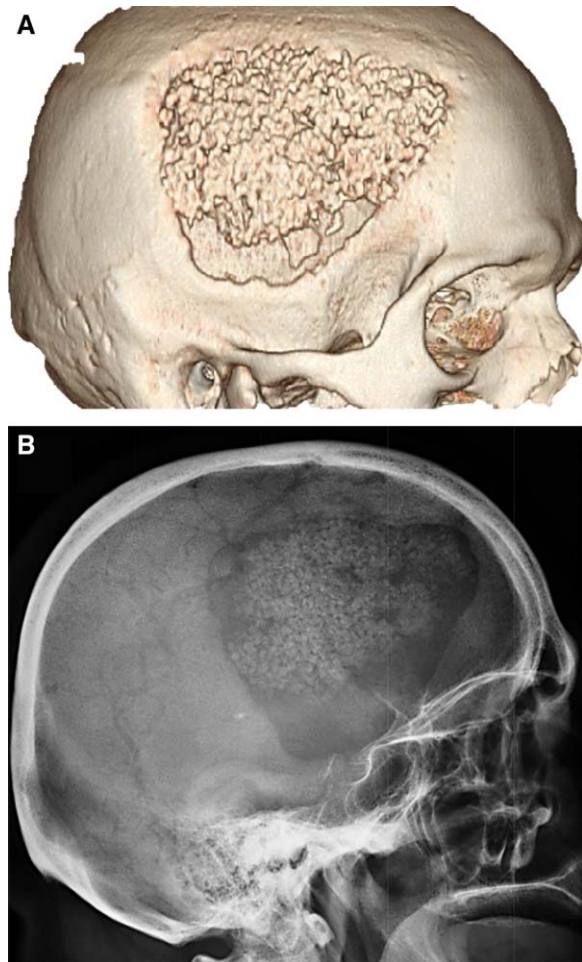
Figure 3. Low-power histological appearance of the biopsy specimen from the skull lesion of patient 1 shows hypocellular degenerated collagenous material and loose fibrinous tissue. Some of the spherical necrotic tissue islands are partially mineralized. Fungus hyphae are present in the fibrous tissue (arrow). There is an inflammatory polymorphonuclear leukocyte infiltrate at the top of the photomicrograph. H&E staining. Original magnification ×40. Scale bar = 100 μm. Abbreviation: H&E, hematoxylin and eosin.

Figure 2. **(A)**: Reformatted CT image of the head 13 months postoperatively (Patient 3). Partial resorption of the graft is seen at the basal area. **(B)**: Skull x-ray 6 years post-operatively (Patient 3) shows substantial resorption of the graft at the borders.

Ethical Considerations

The study was approved by the board of the local hospital district, and Finnish Medicines Agency (FIMEA) was informed about the procedures. Before surgery, the patients were informed about the procedures, which they approved and to which they gave their written consent.

RESULTS

In Vitro Results

The viability and adherence of the ASCs transplanted with the betaTCP granules to the patients was confirmed by Live/Dead staining. The flow cytometry showed that the cells were homogenous in morphology by side-scatter and forward-scatter parameters. However the immunophenotype showed variability between patients. Nevertheless, strong positive expression (>90%) was seen in surface markers CD10, CD13, Cd29, CD44, CD49d, CD73,CD90, CD105, CD106, and CD166. Moderate positive expression (>10%, <90%) was seen for markers CD 9, CD106 and CD146. No expression, or low expression (<10%) was seen for markers CD31, CD34, CD45, and major histocompatibility complex class I isotype human leukocyte antigen DR, suggesting low
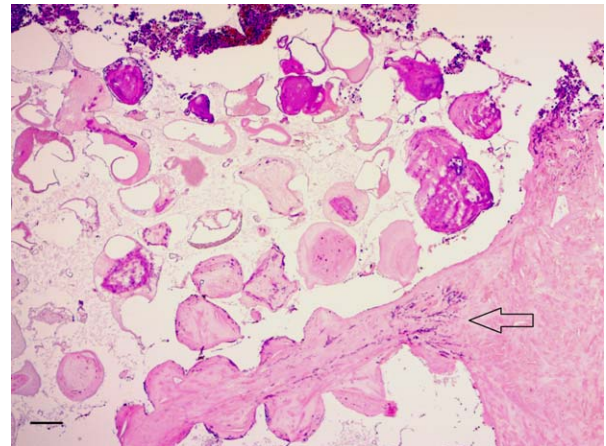
detection of cells of hematopoietic and angiogenic lineages. Moreover, CD14 and CD 19 (hematopoietic markers) showed great variability in surface marker expression, ranging from low to moderate.

According to alkaline phosphatase staining, all patients' cell samples possessed the capacity to differentiate toward the osteogenic lineages.

Clinical Results

During the follow-up period (average 6.6 years, range 6.1–7.4), all patients except one were re-operated. We observed signs of resorption of the graft in three cases out of five (Table 1). One graft (Patient 1) which was surgically explored due to late infection was relatively soft and was supported to a large degree by the titanium mesh. Microbiological cultures of the infected graft were negative and the infection was successfully treated by surgical removal of the graft combined with cefuroxime and levofloxacin (Tavanic, Sanofi, United Kingdom) antibiotics. Two patients had clinical loosening of the graft (Patients 2 and 4). The graft in one patient showed radiological resorption at the borders but the clinical result was good (Patient 3; Fig. 2A, 2B). One patient was re-operated due to recurrence of the meningioma in the dura mater underlying the graft (Patient 5), but the graft as such was successful (Table 1).

Histological Results

A histological sample of the graft was obtained from two re-operated patients. The findings of the graft from Patient 1 were consistent with osteonecrosis and acute to subacute osteomyelitis. Nonviable degenerated tissue with polymorphonuclear inflammatory infiltrates and organizing granulation tissue as well as radiating septate hyphae characteristic of Aspergillus infection were seen (Fig. 3). The graft from Patient 2 showed mainly degenerated osteoid-like tissue islets containing loose collagen and poorly maintained osteoblasts. However, focally there were some well-formed bone trabeculae containing vital osteocytes (Fig. 4). There were no features suggestive of neoplastic change.
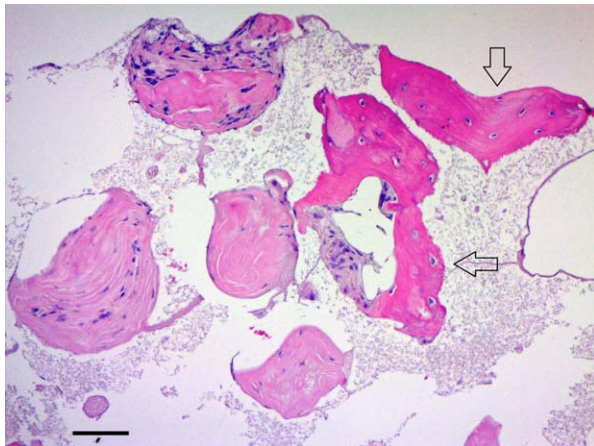
Figure 4. The biopsy specimen from patient 2 shows mainly spherical islands of degenerated collagenous osteoid-like tissue where smudgy appearing nuclei are visible. However, two well-formed trabeculae of woven bone containing viable osteocytes are seen (arrows). H&E staining. Original magnification ×100. Scale bar = 100 μm. Abbreviation: H&E, hematoxylin and eosin.

DISCUSSION

Bone tissue engineering techniques with or without ASCs have been studied actively during past decades [26–28]. ASCs are multipotent cells that are recognized as potentially beneficial in wide variety of medical therapies in reconstructive surgery [27–30]. The potential of these cells has evoked great enthusiasm and high expectations in both scientific and commercial circles. However, in general clinical applications of stem cells in reconstructive surgery have been limited to small case series with varying results and/or short follow-up periods [14, 31–33]. Among the published studies, there is a great variability in, for example, stem cell sources, defect sites and sizes, indications, cell carriers, and use of growth factors that makes it difficult to draw a solid conclusion on stem cell effectiveness in bone tissue engineering [26]. Additionally, there has been concern about the safety and efficacy of stem cells in clinical applications [21, 24, 33, 34].

This long-term follow-up report on five patients receiving cranioplasty using ASCs, betaTCP granules and supporting meshes suggests that the method as such is safe. The short-term results (less than a year) were promising and the bone density of the grafts (measured by HUs) tended to increase [13]. However, in long follow-up, the outcome of the cranioplasties was unsatisfactory in most cases partially due to poor ossification and partially due to infection or tumor recurrence.

Two of the five patients showed marked clinical and radiological resorption of the graft that led to re-operation (Patients 2 and 4). Nevertheless, histology of the re-fixated graft in Patient 2 showed focally well-formed bone trabeculae containing vital osteocytes. One patient had radiological graft resorption, but the graft was firm on palpation, and no re-operation was needed in 7 years follow-up (Patient 3; Fig. 2A, 2B). One patient had a late infection of the graft (>7 years post-operatively) that is not likely to be related to the cranioplasty method (Patient 1). The late infection was most likely due to unsuccessful cranialization of the frontal sinus. Her frontal sinus was opened in the primary operation and filled with artificial material, which may have been the cause of the infection. One patient (Patient 5), who had recurrence of the meningioma 2.2 years after the primary operation,

was found to have a clinically ossified graft that had attached well to defect margins and prevented unfastening of the graft in the re-operation. This patient had a titanium inner mesh, which probably played a role in the success of her case.

There are several questions that must be solved before clinical trials regarding the effectiveness of this strategy are continued. First, how could ossification be induced and enhanced, and how can the bone forming capacity of the ASCs be stimulated in this setup? ASCs themselves may stimulate bone formation not only by differentiating into bone cells but also by secreting paracrine factors that enhance ossification and vascularization. We decided to use ASCs mainly because of their great accessibility and low patient morbidity when compared with other mesenchymal cell sources such as bone marrow or periosteum. The in vitro, in vivo and clinical bone-forming capacity of ASCs in combination with various scaffold materials have been reported by many authors [14, 20, 35–41]. In this series, the flow cytometric characterization of the ASCs expanded in autologous serum corresponded to previously published results for ASCs [42, 43]. According to flow cytometry, and alkaline phosphatase staining results, the cells were of mesenchymal origin and had a capacity to differentiate into the osteoblastic lineage. There was individual variability in the numbers of implanted cells ranging from 28,125 cells/ml to 391,050 cells/ml (average 123,098 cells/ml). In the future, it is vital to find the optimal doses of ASCs and biomaterial granules to achieve effective ossification. Also, the role of growth factors such as rhBMP-2 in reconstructive surgery is indeterminate and safety concerns have emerged in clinical applications [44]. In this series growth factors were not used. The effectiveness of the construct might be further increased by soaking it in venous blood prior to implantation [45, 46]. Moreover, an intact periosteum would be beneficial for bone formation but its role in this setup remains unclear [47].

The purpose of the scaffold in reconstructive tissue engineering is to provide mechanical support for cell proliferation, migration, and activity by mimicking extra cellular matrix [48, 49]. Ideally, the scaffold provides a template for bone formation, and calcium phosphate based materials are considered ideal in this regard. So far, dozens of calcium phosphate materials including betaTCP have been investigated both in vitro and in vivo [50–52]. We used granular betaTCP partly because of its bioresorbability and large surface area compared to solid scaffolds. Granules also molded well to the defects. Moreover, there is prior evidence of the use of betaTCP in craniomaxillofacial surgery [20, 53, 54]. The failure of the graft ossification of Patients 2 and 4 may be due to too rapid resorption of betaTCP granules which in turn may be related to the use of resorbable and less rigid supporting meshes in their cases. In future studies, it is pivotal to find an optimal scaffold material to support ossification in cranial area [55, 56].

The supporting mesh (mono/bilaminate) plays probably a pivotal role in the success of the graft in this setup. The main advantage of a resorbable mesh, such as RapidSorb is that there will be no artificial material left in the graft site in the long run. However, resorbable mesh may lose its strength rather rapidly; for example RapidSorb has 85% strength left after 8 weeks, 40% at 15 weeks, and <10% after 20 weeks [57], which may result in increased micromotion, which potentially prohibits sound bone bridging due to continuous disruption of the fragile initial bone struts and early vessel formation. On the other hand, rapidly resorbing PLGA constructs may accumulate acidity due to hydrolysis of the mesh which in turn may cause hydrolysis of the newly formed mineral

deposits and strong activation of osteoclasts. Based on our current experience, we believe that the use of a rigid mesh such as titanium is necessary for damping dural pulsations to offer optimal circumstances for bone formation when granular scaffold material is used. On the other hand, titanium and other materials may provide surfaces supporting the migration of osteoblasts [58, 59]. In our series, the titanium-containing constructs (Patients 1 and 5) had good intermediate follow-up results and the observed osteolysis was due to non-reconstruction related causes (Patient 1) and recurrent meningioma (Patient 5). In patient 2, a single layer of resorbable mesh may have provided insufficient stability to avoid micromotion sufficiently, hence failure of appropriate integration of graft in time which led to resorption and loosening of the graft. Both Patients 3 and 4 had a double layer of resorbable mesh. The discrepancy of the results between Patient 3 (resorption only at the borders) and Patient 4 (complete resorption of the graft) may be due to markedly higher age of the Patient 4, and lower functionality of her ASCs. However, the limited number of patients and high number of confounding factors makes it impossible to draw solid conclusions in regard to optimal mesh material or technique.

The critical size of the defect for this method remains indeterminate. Large size probably hinders blood supply to central parts of the graft, which may be crucial for bone formation. The defect size in our series was relatively large (average 8.1×6.7 cm^2); however, it was considerably smaller than the average defect size following decompressive hemicraniectomy, which is a common neurosurgical procedure leading to calvarial defects [60, 61]. Obviously, there are several additional issues to consider before the method is taken into clinical use.

CONCLUSION

Conventional cranioplasties have high complication rates, which encourage searching for novel techniques. We performed five cranioplasties using ASCs, betaTCP granules and titanium or resorbable mesh. The 6-year results of most of these cases are unsatisfactory. There were no clear indications that this procedure stimulated ossification or that ACSs or their progeny produced new bone. Nevertheless, there were no serious adverse events, and all patients recovered well from the surgeries. The use of this strategy for cranial defect reconstruction must be studied further before continuing with clinical trials and before applying the method in clinical practice.

AUTHOR CONTRIBUTIONS

T.T.: Conception and design, Collection and/or assembly of data, Provision of study material, Data analysis and interpretation, Manuscript writing, Final approval of manuscript; K.L., T.N.: Conception and design, Provision of study material, Data analysis and interpretation, Manuscript writing, Final approval of manuscript; S.H., B.M., S.M., R.S.-K., J.O.: Provision of study material, Data analysis and interpretation, Manuscript writing, Final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

REFERENCES

1 Feroze AH, Walmsley GG, Choudhri O et al. Evolution of cranioplasty techniques in neurosurgery: historical review, pediatric considerations, and current trends. J Neurosurg 2015;123:1098–1107.

2 Klinger DR, Madden C, Beshay J et al. Autologous and acrylic cranioplasty: a review of 10 years and 258 cases. World Neurosurg 2014;82:e525–e530.

3 Lee CH, Chung YS, Lee SH et al. Analysis of the factors influencing bone graft infection after cranioplasty. J Trauma Acute Care Surg 2012;73:255–260.

4 Sundseth J, Sundseth A, Berg-Johnsen J et al. Cranioplasty with autologous cryopreserved bone after decompressive craniectomy: complications and risk factors for developing surgical site infection. Acta Neurochir (Wien) 2014;156:805–811; discussion 811.

5 Gooch MR, Gin GE, Kenning TJ et al. Complications of cranioplasty following decompressive craniectomy: analysis of 62 cases. Neurosurg Focus 2009;26:E9.

6 Bobinski L, Koskinen LO, Lindvall P. Complications following cranioplasty using autologous bone or polymethylmethacrylate–retrospective experience from a single center. Clin Neurol Neurosurg 2013;115:1788–1791.

7 De Bonis P, Frassanito P, Mangiola A et al. Cranial repair: how complicated is filling a "hole"? J Neurotrauma 2012;29:1071–1076.

8 Sobani ZA, Shamim MS, Zafar SN et al. Cranioplasty after decompressive craniectomy: an institutional audit and analysis of factors related to complications. Surg Neurol Int 2011;2:123.

9 Piitulainen JM, Kauko T, Aitasalo KM et al. Outcomes of cranioplasty with synthetic materials and autologous bone grafts. World Neurosurg 2015;83:708–714.

10 Wachter D, Reineke K, Behm T et al. Cranioplasty after decompressive hemicraniectomy: underestimated surgery-associated complications? Clin Neurol Neurosurg 2013;115:1293–1297.

11 Iaccarino C, Viaroli E, Fricia M et al. Preliminary results of a prospective study on methods of cranial reconstruction. J Oral Maxillofac Surg 2015;73:2375–2378.

12 Williams LR, Fan KF, Bentley RP. Custom-made titanium cranioplasty: early and late complications of 151 cranioplasties and review of the literature. Int J Oral Maxillofac Surg 2015;44:599–608.

13 Thesleff T, Lehtimaki K, Niskakangas T et al. Cranioplasty with adipose-derived stem cells and biomaterial: a novel method for cranial reconstruction. Neurosurgery 2011;68:1535–1540.

14 Sandor GK, Numminen J, Wolff J et al. Adipose stem cells used to reconstruct 13 cases with cranio-maxillofacial hard-tissue defects. STEM CELLS TRANSL MED 2014;3:530–540.

15 Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. Regen Med 2009;4:265–273.

16 Dmitrieva RI, Minullina IR, Bilibina AA et al. Bone marrow- and subcutaneous adipose tissue-derived mesenchymal stem cells: differences and similarities. Cell Cycle 2012;11:377–383.

17 Dai R, Wang Z, Samanipour R et al. Adipose-derived stem cells for tissue engineering and regenerative medicine applications. Stem Cells Int 2016;2016:6737345.

18 Mizuno H, Tobita M, Uysal AC. Concise review: adipose-derived stem cells as a novel tool for future regenerative medicine. STEM CELLS 2012;30:804–810.

19 Schaffler A, Buchler C. Concise review: adipose tissue-derived stromal cells–basic and clinical implications for novel cell-based therapies. STEM CELLS 2007;25:818–827.

20 Mesimaki K, Lindroos B, Tornwall J et al. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg 2009;38:201–209.

21 Eaves FF,3rd, Haeck PC, Rohrich RJ. ASAPS/ASPS Position statement on stem cells and fat grafting. Plast Reconstr Surg 2012;129:285–287.

22 Munsie M, Hyun I. A question of ethics: selling autologous stem cell therapies flaunts professional standards. Stem Cell Res 2014;13:647–653.

23 Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol 2014;32:252–260.

24 Heslop JA, Hammond TG, Santeramo I et al. Concise review: workshop review: understanding and assessing the risks of stem cell-based therapies. STEM CELLS TRANSL MED 2015;4:389–400.

25 European Pharmacopoeia. Chapter 2.6.1, membrane filtration and Chapter 2.6.14,

kinetic chromogenic LAL method. In: European Pharmacopoeia. 6th ed. Strasbourg: Council of Europe, 2007:155–159, 182–189.

26 Khojasteh A, Behnia H, Dashti SG et al. Current trends in mesenchymal stem cell application in bone augmentation: a review of the literature. J Oral Maxillofac Surg 2012;70:972–982.

27 Yousefi AM, James PF, Akbarzadeh R et al. Prospect of stem cells in bone tissue engineering: a review. Stem Cells Int 2016; 2016:6180487.

28 Salibian AA, Widgerow AD, Abrouk M et al. Stem cells in plastic surgery: a review of current clinical and translational applications. Arch Plast Surg 2013;40:666–675.

29 Gir P, Oni G, Brown SA et al. Human adipose stem cells: current clinical applications. Plast Reconstr Surg 2012;129:1277–1290.

30 Weyand B, Vogt PM. Potential of mesenchymal stem cell applications in plastic and reconstructive surgery. Adv Biochem Eng Biotechnol 2013;130:55–67.

31 Behnia H, Khojasteh A, Soleimani M et al. Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: a preliminary report. J Craniomaxillofac Surg 2012;40:2–7.

32 Sandor GK, Tuovinen VJ, Wolff J et al. Adipose stem cell tissue-engineered construct used to treat large anterior mandibular defect: a case report and review of the clinical application of good manufacturing practice-level adipose stem cells for bone regeneration. J Oral Maxillofac Surg 2013;71:938–950.

33 Kozlik M, Wojcicki P. The use of stem cells in plastic and reconstructive surgery. Adv Clin Exp Med 2014;23:1011–1017.

34 Main H, Munsie M, O'Connor MD. Managing the potential and pitfalls during clinical translation of emerging stem cell therapies. Clin Transl Med 2014;3:10.

35 Conejero JA, Lee JA, Parrett BM et al. Repair of palatal bone defects using osteogenically differentiated fat-derived stem cells. Plast Reconstr Surg 2006;117:857–863.

36 Dragoo JL, Lieberman JR, Lee RS et al. Tissue-engineered bone from BMP-2-transduced stem cells derived from human fat. Plast Reconstr Surg 2005;115:1665–1673.

37 Waselau M, Patrikoski M, Juntunen M et al. Effects of bioactive glass S53P4 or beta-tricalcium phosphate and bone morphogenetic protein-2 and bone morphogenetic protein-7 on osteogenic differentiation of human adipose stem cells. J Tissue Eng 2012; 3:2041731412467789.

38 Levi B, James AW, Nelson ER et al. Human adipose derived stromal cells heal critical size mouse calvarial defects. PLoS One 2010;5:e11177.

39 Pei M, Li J, McConda DB et al. A comparison of tissue engineering based repair of calvarial defects using adipose stem cells from normal and osteoporotic rats. Bone 2015;78: 1–10.

40 Tirkkonen L, Haimi S, Huttunen S et al. Osteogenic medium is superior to growth factors in differentiation of human adipose stem cells towards bone-forming cells in 3D culture. Eur Cell Mater 2013;25:144–158.

41 Lendeckel S, Jodicke A, Christophis P et al. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. J Craniomaxillofac Surg 2004;32:370–373.

42 Strem BM, Hicok KC, Zhu M et al. Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 2005;54:132–141.

43 Shahdadfar A, Fronsdal K, Haug T et al. In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. Stem Cells 2005;23:1357–1366.

44 James AW, LaChaud G, Shen J et al. A review of the clinical side effects of bone morphogenetic protein-2. Tissue Eng Part B Rev 2016;22:284–297.

45 Wang X, Friis T, Glatt V et al. Structural properties of fracture haematoma: current status and future clinical implications. J Tissue Eng Regen Med 2016. doi: 10.1002/term. 2190. [Epub ahead of print]

46 Shiu HT, Goss B, Lutton C et al. Formation of blood clot on biomaterial implants influences bone healing. Tissue Eng Part B Rev 2014;20:697–712.

47 Wang T, Zhang X, Bikle DD. Osteogenic differentiation of periosteal cells during fracture healing. J Cell Physiol 2017;232:913–921.

48 Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol 2012;30:546–554.

49 Padial-Molina M, O'Valle F, Lanis A et al. Clinical application of mesenchymal stem cells and novel supportive therapies for oral bone regeneration. Biomed Res Int 2015; 2015:341327.

50 Cameron HU. Tricalcium phosphate as a bone graft substitute. Contemp Orthop 1992; 25:506–508.

51 Kamakura S, Sasano Y, Shimizu T et al. Implanted octacalcium phosphate is more resorbable than beta-tricalcium phosphate and hydroxyapatite. J Biomed Mater Res 2002; 59:29–34.

52 Hollinger JO, Battistone GC. Biodegradable bone repair materials. Synthetic polymers and ceramics. Clin Orthop Relat Res 1986; (207):290–305.

53 Gorla LF, Spin-Neto R, Boos FB et al. Use of autogenous bone and beta-tricalcium phosphate in maxillary sinus lifting: a prospective, randomized, volumetric computed tomography study. Int J Oral Maxillofac Surg 2015;44:1486–1491.

54 Zijderveld SA, Zerbo IR, van den Bergh JP et al. Maxillary sinus floor augmentation using a beta-tricalcium phosphate (Cerasorb) alone compared to autogenous bone grafts. Int J Oral Maxillofac Implants 2005;20:432–440.

55 Zanotti B, Zingaretti N, Verlicchi A et al. Cranioplasty: review of materials. J Craniofac Surg 2016;27:2061–2072.

56 Khader BA, Towler MR. Materials and techniques used in cranioplasty fixation: a review. Mater Sci Eng C Mater Biol Appl 2016; 66:315–322.

57 RapidSorb rapid resorbable fixation system. Available at http://synthes.vo.llnwd.net/o16/LLNWMB8/US%20Mobile/Synthes%20North%20America/Product%20Support%20Materials/Technique%20Guides/DSUSBIO01150058_RAPIDSORB.pdf. Accessed 2017.

58 Giannitelli SM, Accoto D, Trombetta M et al. Current trends in the design of scaffolds for computer-aided tissue engineering. Acta Biomater 2014;10:580–594.

59 Murphy WL, McDevitt TC, Engler AJ. Materials as stem cell regulators. Nat Mater 2014;13:547–557.

60 Wagner S, Schnippering H, Aschoff A et al. Suboptimum hemicraniectomy as a cause of additional cerebral lesions in patients with malignant infarction of the middle cerebral artery. J Neurosurg 2001;94:693–696.

61 Huttner HB, Schwab S. Malignant middle cerebral artery infarction: clinical characteristics, treatment strategies, and future perspectives. Lancet Neurol 2009;8:949–958.