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Case Report

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First Experience of Personalized *in Situ* Tissue Engineering for Thoracic Surgery of the Sarcoma Patient: MSCs-Containing Minimally Manipulated Cells and an Individualized Micropore Titanium Sternum in a One-Year Follow-Up Case Report

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ABSTRACT

Individually customized grafts have become standard for reconstructing extensive chest wall defects resulting from surgical interventions for sternal malignant neoplasms. However, the outcomes of these graft implantations can be further improved by administering patient-derived cells, which have minimal oncological risks. In 2021, a 52-year-old woman with chondrosarcoma (pT2N0M0G2, stage IIB) was admitted to the Department of Thoracic Surgery. The patient presented with a large tumor in the body of the sternum, measuring 81 × 94 × 91 mm, according to the computed tomography (CT) scan. To address this, an individualized endoprosthesis was modeled and created using the original 'pincer-dock' construction based on CT-scan screens. The mononuclear cell fraction (MNCs) was obtained from the patient's peripheral blood one week before surgery using a Haemonetics cell separation device and cryopreserved until the day of the procedure. The resulting 30 mL MNC suspension contained 12 mln cells per 1 mL. We performed flow cytometry analysis using a FACS Aria III flow cytometer to confirm the presence of mesenchymal stromal cells in the MNCs. We also performed immunostaining for S-100, a common tumor marker for benign and malignant diseases, and D2-40, a marker for the lymphatic endothelium that reacts with Kaposi's sarcoma and a subset of angiosarcomas. None of the cells were positive for either marker. Approximately 3 ml of the MNC suspension was injected into each rib edge and 30 ml into the operating field immediately after resection. The titanium endoprosthesis was placed in the sternal defect, and the body of the endoprosthesis was securely covered with a laparoscopically mobilized omental flap. After a one-year follow-up, the patient showed no signs of recurrence or post-surgical complications. These outstanding functional and cosmetic results highlight the potential for the broader clinical utilization of minimally manipulated cells in personalized medicine in oncology. These results could pave the way for wider clinical application of peripheral blood-derived minimally manipulated cells in personalized medicine as an adjuvant for titanium endoprosthesis reconstruction of osteochondral defects in patients with sarcoma.

Keywords: Endoprosthesis; Minimally manipulated cells; Regenerative medicine; Sarcoma; Stem cell transplantation; Tissue engineering

INTRODUCTION

Individually customized grafts have recently become the gold standard for the primary or secondary reconstruction of extensive chest wall defects that occur after surgical intervention for sternal malignant neoplasms[1,2]. Surgeons typically use carbon and titanium grafts with appropriate biocompatibility and acceptable cosmetic properties[3]. Most grafts are made of microporous titanium fabricated by layer-to-layer metal powder fusion. The microporous structure is expected to enhance bone ingrowth and graft integration. Despite the advantages of microporous titanium, bone destruction has been reported in numerous cases involving massive engrafting. These complications lead to false rib-to-rib joints and local inflammation, pain, and physical disability[4]. The main causes of long-term complications are graft malfunction, anastomosis failure caused by insufficient vascularization, and upregulated chronic inflammation. Controllable upregulation of the local tissue regeneration capacity is required to ensure superior biointegration of massive grafts. The topical application of cells on biocompatible matrices for local tissue remodeling is an *in situ* tissue engineering approach[5].

In situ tissue engineering using minimally manipulated cells is a promising approach to reduce cellular stress and avoid the impact of expansion in cell culture[5,6]. These features of minimally manipulated cells may reduce adverse events for high-risk patients in oncological practice that are caused by tumor-originating and poorly differentiated cells[7]. Therefore, minimally manipulated cells can be used to seed various grafts in high-risk reconstructive surgeries. In this case report, we implanted a personalized micropore titanium sternum enriched with minimally manipulated mononuclear cells.

Case presentation

In 2021, a 52-year-old woman with chondrosarcoma (pT2N0M0G2, stage IIB) was admitted to the Department of Thoracic Surgery. The patient had a massive tumor localized in the body of the sternum, measuring up to 81 × 94 × 91 mm, according to the computed tomography (CT) scan. The tumor involved the S5 segment of the right lung and disrupted the xiphoid process in the sternum. Histological analysis confirmed a diagnosis of chondrosarcoma (grade II). The tumor board recommended extended sternal resection with combined primary reconstruction using personalized titanium endoprosthesis autologous soft tissues for vascularization and cell therapy with minimally manipulated cells for superior biointegration of the massive graft. Mononuclear cells improve bone reconstruction using autologous bone or metal grafts. Long-term results can be improved primarily by the presence of mesenchymal stem cells (MSCs) and a subpopulation of cluster of differentiation CD34+ cells. Both can stimulate osteoblastic differentiation and vascularization[8].

The emerging concept of minimally manipulated cells centers on cell processing without substantial manipulation and does not require special Good Manufacturing Practice (GMP) facilities for preparation. In accordance with Russian legal regulations, minimally manipulated cells have been accepted recently as objects for autotransplantation. They can be administered in routine clinical practice, granting fast-track access to regenerative medicine[9]. Mononuclear cells (MNCs) were separated from the patient's peripheral blood one week prior to surgical treatment using a Haemonetics cell separation device (Haemonetics, USA) and cryopreserved until the day of surgery. The resulting 30 mL MNC suspension contained 12 mln cells per 1 mL. We performed flow cytometry analysis using a FACSAria III flow cytometer to confirm the presence of MSCs and CD34+ cells in the MNCs. The positive impact of MSCs on osteoplastic surgery has been described in an extended list of clinical studies and is explained by their capability to participate in bone healing[10]. Commonly accepted criteria for recognizing poorly differentiated multipotent MSCs include positive labeling for CD73, CD105, and CD90 and negative labeling for CD45. However, MSCs can differ in CD90 expression depending on their differentiation status[11]. Furthermore, an extended analysis of non-cultured bone marrow-resident MSCs has shown that they exist in CD34-dim or positive fractions[12].

Therefore, we formulated the following MSC identification criteria: CD34-dim/positive and side scatter (SSC)-high gated cells were also positive for CD73, CD90, and CD105. We observed more than 1% CD73+; however, only 1.3% were identified as MSCs embracing 0,3% of the total MNCs (Figure 1A). We also observed a heterogeneous population of stem cells and endothelial progenitors by gating a well-defined population of CD34+ cells (0.1% of the total MNCs) (Figure 1B). Several clinical trials and described cases have shown that applying CD34+ cells promotes bone tissue regeneration[13].

For patient safety reasons, we performed immunostaining for S-100, a common tumor marker for both benign and malignant diseases, and D2-40, a marker of the lymphatic endothelium that reacts with Kaposi's sarcoma and a subset of angiosarcomas. None of the cells was positive for either marker.

The individual endoprosthesis was modeled according to our original 'pincer-dock' construction, described previously[14]. The endoprosthesis was cold-printed with titanium powder, resulting in a microporous structure. The volumetric body had perforated 'pincer' connections with movable upper parts, enabling atraumatic rib grasping for fixation.

We performed sternal resection and crosscut the second to sixth rib pairs. Atypical resection of the lower lobe of the right lung was performed. The titanium sternum was fixed to the edges of the resected ribs and manubrium by clamping the ribs to the pincers. Fixation sites were enriched with autologous, minimally manipulated MNCs via injection at the rib edges and sternum (Figure 1C). Approximately 3 ml of MNC suspension was injected into each rib edge, resulting in total consumption of 30 ml. The large pectoral muscles were sutured to the endoprosthesis. Finally, the endoprosthetic body was wrapped in a laparoscopically mobilized omental flap (Figure 1D).

The patient was discharged from our clinic nine days after surgery. The titanium sternum did not cause pain over more than 4 points on the visual analog scale during the first year of follow-up; therefore, the patient was physically active as usual and could return to her regular quality of life. We performed chest CT at 3 and 6 months after surgery. Both screenings revealed that the graft held the same stable position with no signs of inflammation, migration, or deformation (Fig. 2). Excellent biointegration and neo-formed high-density bone tissue were observed on the rib edges inside the clamps. Despite massive metal engrafting, the surgery has yielded excellent functional and cosmetic results.

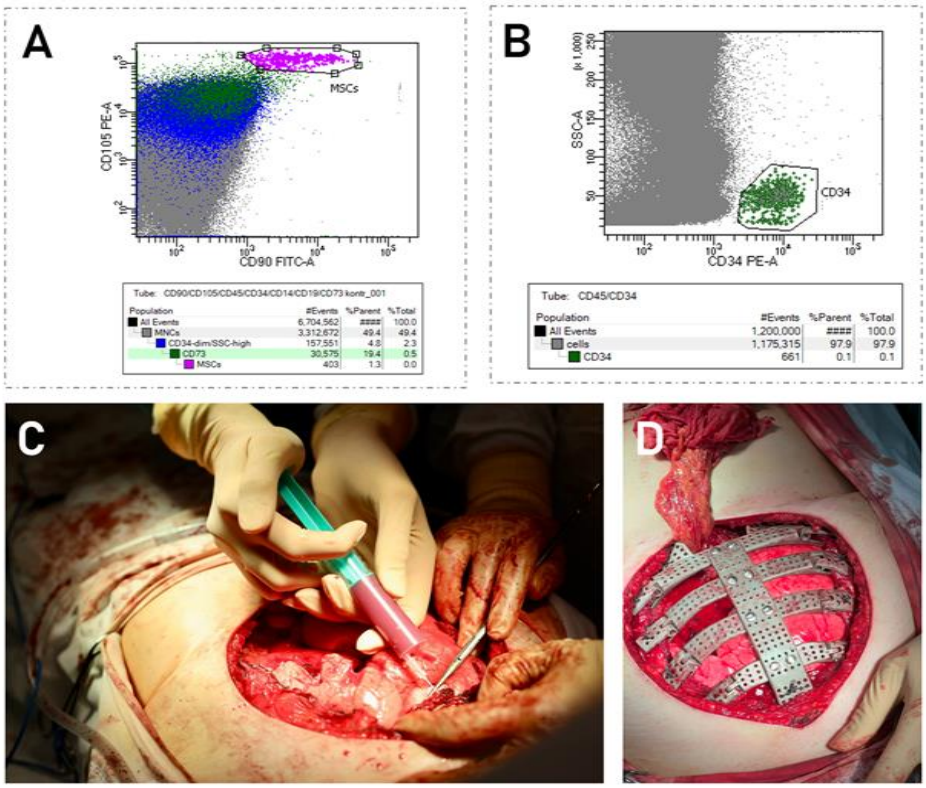

Figure 1. MNCs flow cytometry and tissue engineering *in situ*: (A) flow cytogram of the MNC suspension, violet-colored and encircled - population of MSCs; (B) flow cytogram of MNC suspension, green-colored and encircled - subpopulation of CD34+ heterogeneous stem cells; (C) intraoperative injection of cell suspension in the sternal resection edge; (D) individualized titanium sternum on place: securing crab clips for fixation, and the omental flap is visible as extracted and prepared for covering. All images are published in accordance with the patient's consent

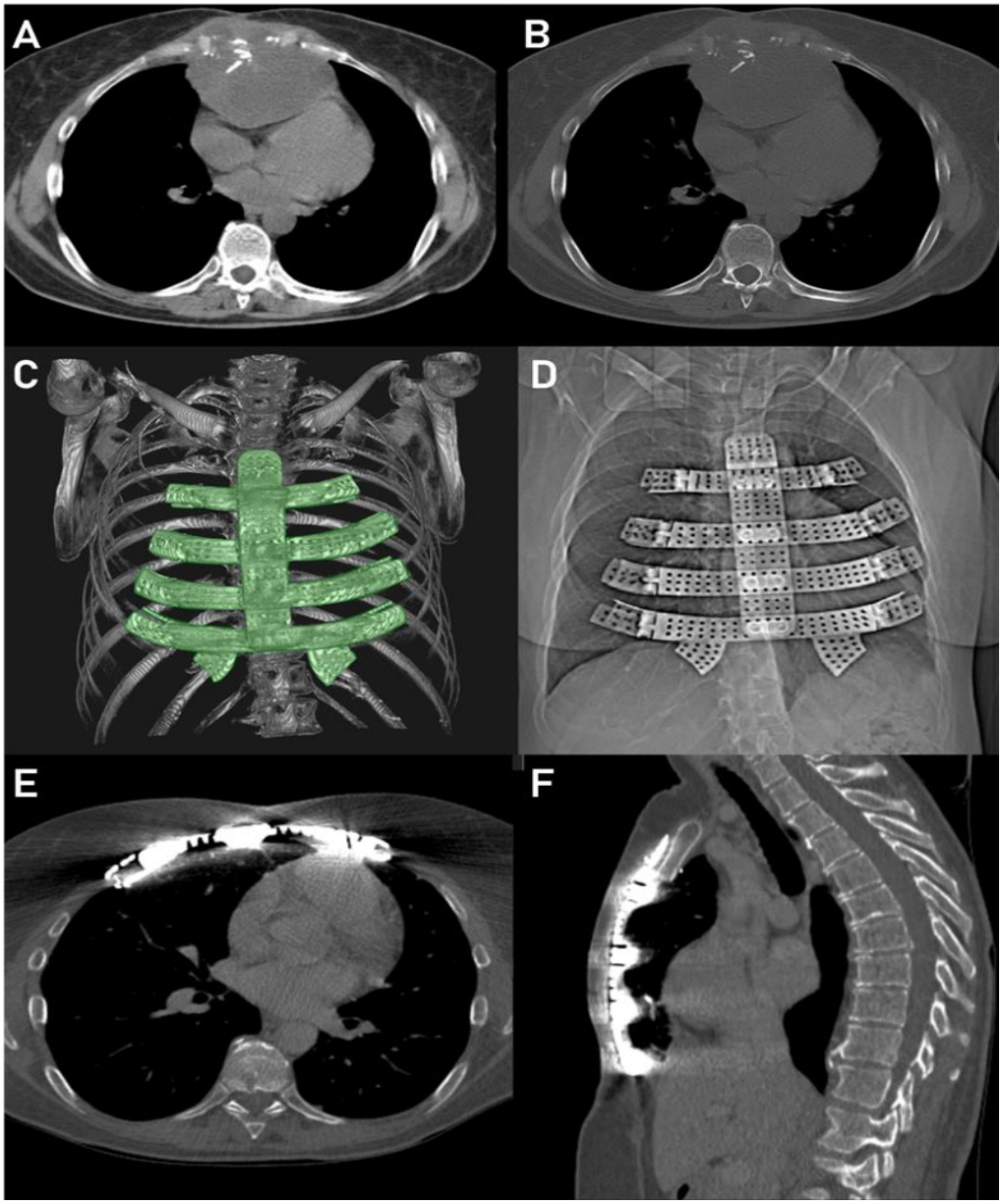

Figure 2. Thorax CT: (A) axial view soft tissue window, and (B) bone window, shows the massive tumor located in the *Manubrium sternum* with retrosternal expansion; (C) three-dimensional view, and (D) CT topogram, (E) axial view, and (F) sagittal view shows the tissue-engineered titanium sternum six months after surgery; All images are published in accordance with the patient's consent

DISCUSSION

Sternal sarcomas are quite rare cases compared to indications that require sternal resection, with the majority of chest wall neoplasms being breast cancer or metastatic lesions[15]. However, up to 82% of patients requiring sternal prosthetic reconstruction are diagnosed with sternal sarcoma[16]. Attempts to replace the sternum with an omental flap commonly result in reduced quality of life due to pain and herniation[17]. Titanium grafts have demonstrated better clinical outcomes than all other available materials[18]; however, the outcomes have individual features and may lead to long-term complications. Nevertheless, extended sternal resection cannot avoid extensive mobilization of soft tissues (i.e., pectoral muscles) and multiple sites of bone crosscuts[19]. Multiple sites of injury caused by surgical aggression exceed the limits of the local endogenous regenerative potential. *In situ* tissue engineering can overcome the risk of complications via intraoperative remodeling of the cell niche (such as microporous materials and growth factors) or local administration of cell therapy. Furthermore, *in situ* tissue engineering implies the application of cells directly in the operating theater. In contrast, traditional tissue engineering requires a long exposure time for cell seeding and maturation and entails potential risks related to cell expansion. We previously discussed this challenging concept as the most promising for implanting massive grafts [5]. We previously showed that microporous titanium with a developed surface could be effectively seeded with bone marrow-derived MNCs[20].

Therefore, moving towards *in situ* tissue engineering, we prepared a novel tissue-engineered sternum combined with minimally manipulated cells and individually printed grafts. We used immunostaining for tumor markers to ensure the absence of circulating tumor cells in the minimally manipulated cell suspension. We propose our approach adheres to the highest safety standards for cell therapy in oncology, minimizing cell-related risks to the patient. Furthermore, polymerase chain reaction could accompany future clinical cases for precise genetic analysis of tumor-related cells. Our positive functional and superior cosmetic results during the one-year follow-up period could pave the way for wider clinical applications of minimally manipulated cells in personalized medicine.

Statements

The authors declare that this study has not been published before and is not being considered for publication elsewhere in its final form.

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Statement of Ethics

The trial was approved by the ethics committee of the National Medical Research Radiological Center (No. 793 issued on March 11, 2022). Written informed consent was obtained from the patient for all published pictures and data in this case report.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding the subject discussed in this case report.

Author Contributions

VSU, GVA, and DSB operated on the patient; IVK guided the manufacture of the endoprosthesis, conceived and supervised the surgery and postoperative treatment; AYU, SSS, DSB, and IDK derived the cells; LYG, DSB, and IDK provided flow cytometry analysis; DOK and NAR performed the radiology study; DSB, IDK, and OAA wrote the manuscript; and PVS, SAI, and ADK approved the

final version to be published. All authors revised and approved the final draft of the manuscript.

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