Undifferentiated mesenchymal stem cells seeded on a vascular prosthesis contribute to the restoration of a physiologic vascular wall

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Background: We evaluated the possibility of restoring a physiologic vascular wall using undifferentiated mesenchymal stem cells (MSCs) seeded on a polyurethane vascular prosthesis.

Methods: Undifferentiated MSCs were seeded on a vascular prosthesis and implanted into Wistar male rats (weight, 350 g) to investigate differentiation into smooth muscle cells and to determine graft endothelialization in vivo.

Results: Seeded or nonseeded grafts were surgically implanted. Undifferentiated MSCs were first labelled for green fluorescent protein. After 2 weeks in vivo, MSC that were initially self-expanded on the graft in a monolayer were organized in a multicellular layer mimicking media of aortic adjacent wall. They coexposed green fluorescent protein and smooth muscle proteins that were not present before the in vivo engraftment, indicating that in vivo conditions induced smooth muscle protein maturation. Undifferentiated MSC showed an electrophysiologic profile quite different than mature smooth muscle cells. In both in vitro- and in vivo-differentiated MSCs, adenosine triphosphate, an IP3-dependent agonist, induced an increase in calcium similar to that which occurred in mature smooth muscle cells. However, MSCs failed to respond to caffeine, a ryadonide receptor activator, indicating the absence of mature calcium signaling, and finally, contraction was absent. Endothelialization attested by immunohistology and scanning electron microscopy was greater in MSC-seeded grafts that prevent thrombosis.

Conclusion: Only partial smooth muscle cell differentiation of MSCs resulted when seeded on vascular grafts, but MSCs spontaneously restore a media-like thick wall. Mesenchymal stem cells have a positive impact on in vivo endothelialization in rats that supports their potential for use in vascular surgery.

Clinical Relevance: Thrombosis of vascular prostheses is a major complication of surgery. We showed on rat aorta that mesenchymal stem cells seeded on polyurethane patch restore endothelium. It also induced incomplete smooth muscle differentiation. In the future, stem cell could prevent thrombosis of vascular prostheses. (J Vasc Surg 2008;47:1313-21.)

Despite technologic advances in biomaterials, the surgical impact of small-sized vascular prostheses in peripheral vascular bypass surgery has been poor, with a positive outcome reported as low as 50% at the 1-year follow-up. Bioengineering of vascular grafts using stem cells could promote endothelialization, thus preventing thrombosis. To this end, several studies have attempted to cover the graft directly using endothelial cells. In 1984 Herring et al described, for the first time, the use of endothelial cells to cover vascular grafts. Endothelialization was observed more often with polytetrafluoroethylene (PTFE) than with a Dacron graft. However, seeded grafts were covered largely by fibroblasts. Bhattacharya et al showed that CD34+ cell seeding enhances vascular graft endothelialization in a dog model. These promising results were overshadowed, however, by the difficulty in obtaining high-quality coverage of grafts. Furthermore, endothelial cells detached when grafts were exposed to in vitro shear stress corresponding to in vivo hemodynamic conditions.

Although collagen coating improves primary cellular adhesion, the degree of spreading depends on the underlying surface structure and on the application of shear stress. Recent procedures involved the pretreatment of prosthesis material with fibrin, collagen, or other matrix molecules that promote cell attachment and retention, but clinical relevance of these biomaterials have to be confirmed by large, human studies.

Endothelial progenitors alone or cocultured with myofibroblasts and fibrin preparations give also promising results. Mesenchymal stem cells (MSCs) contribute to angiogenesis in the bone marrow and support hematopoietic cells in adult bone marrow. Because of their multipotent nature, MSCs can maintain cellular plasticity and can differentiate toward several phenotypes. They are defined by their adipose, cartilaginous, and osteogenic differentiation capacity. Their pluripotency also includes the ability to develop into a vascular component. This vascular differentiation has only been demonstrated in vitro with human and mouse MSCs, however, and no study has clearly shown this type of differentiation in vivo.

These fibroblast-like cells exhibit adherence, expansion, and resistance properties conducive to grafting and allow the transacted by genes to enhance local nitric oxide
production.\textsuperscript{14} We therefore hypothesized that undifferentiated MSCs seeded on a vascular prosthesis could differentiate into smooth muscle cells (SMCs) or endothelial cells alike and contribute to partial wall restoration. Only a few studies have focused on the use of MSCs for graft engineering.\textsuperscript{15} In the study of Cho et al,\textsuperscript{16} MSCs first differentiated into SMCs or endothelial cells as before implantation. This is time-consuming with some restrictions for clinical use. Hashi et al\textsuperscript{17} used biodegradable nonfibrous scaffold with MSCs to prevent thrombosis and vascular remodeling.

In the present study, we focused on the use of undifferentiated cells seeded on vascular grafts implanted in the rat aorta. We first studied the quality of SMC differentiation by exploring MSC functional proprieties to determine if MSCs allowed restoring a physiologic vascular wall. Finally, we evaluated the quality of the endothelial covering after 1 to 2 weeks in vivo compared with nonseeded grafts.

**MATERIAL AND METHODS**

**Isolation of mesenchymal stem cells.** Cell isolation and culture procedures for MSCs have been established and published previously.\textsuperscript{18-20} Briefly, femurs were aseptically harvested from 6- to 8-week-old Wistar rats, and the adherent soft tissue was removed. Whole marrow plugs were harvested from 6- to 8-week-old Wistar rats, and the adherent cells were separated under fluorescent microscopy before implantation. This is time-consuming with some restrictions for clinical use. Hashi et al\textsuperscript{17} used biodegradable nonfibrous scaffold with MSCs to prevent thrombosis and vascular remodeling.

In the present study, we focused on the use of undifferentiated cells seeded on vascular grafts implanted in the rat aorta. We first studied the quality of SMC differentiation by exploring MSC functional proprieties to determine if MSCs allowed restoring a physiologic vascular wall. Finally, we evaluated the quality of the endothelial covering after 1 to 2 weeks in vivo compared with nonseeded grafts.

**Graft seeding with mesenchymal stem cells.** Undifferentiated MSCs were labeled with green fluorescent protein (GFP) after stable viral gene transfection with LNCX-GFP vector at the first passage, as previously described.\textsuperscript{20} Passage two MSCs were seeded on a vascular prosthesis at a density of \(10^6/cm^2\), and grafts were maintained in culture growth medium composed of Modified Eagle Medium Alpha (MEM; Invitrogen Corporation, Carlsbad, Calif) supplemented with 20% fetal calf serum (FCS; HyClone, Logan, Utah), with an antibiotic solution (1% penicillin/streptomycin; Invitrogen) and an antimitotic solution (0.01% amphotericin B; Bristol-Myers, New York, NY). The marrow plugs were dispersed to obtain a single-cell suspension by sequentially passing the dispersion through 18- and 22-gauge needles. The cells were centrifuged and resuspended with culture medium. Adherent second passage cells were analyzed by flow cytometry and were positive for CD90 and CD73, and negative for CD45, confirming that only MSCs had been selected.

**Animal protocols.** All animal investigations were done accordance with the Guide for the Care and Use of Laboratory Animals (United States National Institutes of Health, NIH Publications 85-23, revised 1996), with European Directives (86/609/CEE), and approved by the local ethical committee (CREAA).

Thirty-six Wistar male rats (weight, 350 g) were randomly separated into two groups and received either seeded or nonseeded grafts (Vascular-Patch, Braun, Tutlingen, Germany). The first group of rats (\(n = 12\)) was used to assess the in vivo differentiation of MSCs into SMCs after 2 weeks in vivo, and the second group (\(n = 24\)) was studied for endothelialization. Within this group, 12 rats received a seeded graft and were euthanized after 1 week (\(n = 6\)) or 2 weeks (\(n = 6\)), and 12 other rats were implanted with nonseeded grafts and were euthanized at 1 week (\(n = 6\)) or 2 weeks (\(n = 6\)) after implantation.

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**Surgical procedure.** Animals were anesthetized with ketamine (100 mg/kg; Virbac, Carros, France) and larcagyl (5 mg/kg; Haupt Pharma, Livron, France) by intraperitoneal injection. After median laparotomy, digestive loops were displaced and the left edge of the aorta was dissected. Collateral vessels were ligatured and a longitudinal aortotomy was performed to allow the suture of a 1-cm long \(2 \times 2\) mm wide prosthesis beyond the renal arterial bifurcation. Neither anticoagulation nor antiaggregate treatment was administrated after recovery.

All rats survived despite the temporary interruption in intestinal transit during the first days after intervention. Doppler imaging using a high frequency probe was used to assess the graft potency before the animals were euthanized (Sequoia Acuson C256 ultrasonographic system; Mountain View, Calif).

**In vivo assessment of smooth muscle cell differentiation.** In the first group of rats (\(n = 12\)), a part of the prosthesis was analyzed by immunohistology using a FluorView500 confocal microscope (Olympus, Melville, NY) with a FluoView500 algorithm. Seeded cells were detached from the second part of the prosthesis after trypsin digestion. These cells were plated in LabTek (Nunc A/S, Roskilde, Denmark) in a culture growth environment. At confluence, the cells were either fixed and tested for smooth muscle-specific protein expression by immunohistologic staining or placed in standard growth medium and used for an electrophysiologic and functional study (patch clamp, calcium florescence, and contraction) \(\leq 1\) week.

The smooth muscle protein expression profile (α-smooth muscle actin [SMA], desmin, smooth muscle myosin heavy chain [SMMHC], myosin light chain-2 [MLC2]) was compared among undifferentiated MSCs and in vivo-differentiated MSCs. Cells were incubated with a primary antibody against rat α-SMA, rat desmin, against rat SMMHC, or rat MLC2 (all from Santa Cruz Biotechnology, Santa Cruz, Calif) at 4°C for 12 hours. The antigen–antibody reaction was detected using a molecular probe Alexa fluor dye specific secondary or phycoerythrin antibody (Interchim, Montlucon Cedex, France) and the positive reaction was visualized by fluorescent microscopy. The colocalization with GFP fluorescence was researched.

Reverse transcription–polymerase chain reaction (RT-PCR) was also performed to assess smooth muscle proteins encoding genes (α-SMA, desmin, SM-22a, MMHC-11) expression. The total RNA of rat MSC was isolated using TRizol (Invitrogen Gibco-BRL), followed by treatment with DNase. Reverse transcription was performed using the RT system Platinum Taq (Invitrogen). RNA (100 ng) was used in the reaction, and random hexamer primers were
used for complimentary DNA (cDNA) synthesis. After the RT procedure, the reaction mixture (cDNA) was used for the PCR. The PCR products were electrophoresed through a 1% agarose gel, and the amplified cDNA bands were visualized by ethidium bromide staining.

The following were used for the functional assessment of the MSCs:

**Patch-clamp experiments.** Electrophysiologic recordings were obtained using the conventional patch-clamp technique in the whole cell configuration. Results for MSCs were compared with isolated carotid and aortic arterial cells. Cells were placed in a 0.5 mL volume bath and continuously superfused by gravity with physiologic saline solution (PSS) at a rate of 1 mL/min. Cell membrane currents were recorded with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Union City, Calif.). The net macroscopic currents were generated by stepwise 10-mV depolarizing pulses (400 ms duration, 5-second intervals) with a constant holding potential of –60 mV from –100 to +100 mV and normalized to the cell capacitance, then expressed in current density. Response to K+ channels blockers was recorded after adding an aliquot of iberiotoxin to a final concentration of 100 nM in the bath and 4-aminopyridine (3mM) to block the BKCa (20) and Kv channel types (32), respectively. Both iberiotoxin and 4-aminopyridine were provided by Sigma (Sigma, St-Quentin Fallaire, France). Voltage-clamp protocols were generated and the data were captured with a computer using a Digidata 1200 interface and pClamp8 software (both Axon Instruments). The analysis was done with Clampfit 8.1 and Origin 6.0 software (Microcal Software, Northampton, Mass).

**Intracellular Ca2+ measurement.** Mesenchymal stem cells were plated on coverslips and loaded with the Ca2+-sensitive fluorescent probe, Fura-2 AM (2 μM; 45 minutes at room temperature). [Ca2+]i was estimated from the Fura 2-AM fluorescence using dual-wavelength excitation (340 and 380 nm) and single emission (510 nm). The recording system included a Nikon Diaphot inverted microscope fitted with epifluorescence (Nikon France, Charenton-le-Pont, France). The intensities of the transmitted light were recorded by one photometer, and single photon currents were converted to voltage signals. Signals at each wavelength were digitized and stored on a personal computer using a PC-Lab Card 812PG interface (Advantech, Nanterre, France). The fluorescence ratio (340/380) was calculated online and displayed with the two voltage signals on a monitor.

**In vitro contraction of mesenchymal stem cells.** Mesenchymal stem cells were plated in a specific glass chamber in the presence of PSS and observed after attachment to an optical inverted microscope (Olympus IX50) with a charge-coupled device camera that allowed images to be recorded continuously. A pipette containing either ATP (10^{-4} mol/L) or potassium chloride (80 mmol/L) was used with a micromanipulator to prevent the induction of mechanical stress. A small amount of solution was injected directly onto the cell membrane. Cells were observed before and after drogues (ATP and potassium chloride) infusion.

**Endothelialization evaluation.** In the second group of rats (n = 24), the collected prostheses were divided into two parts and used for immunohistologic or electron microscopic analysis. We evaluated the expression of the specific endothelial markers, CD31 (platelet/endothelial cell adhesion molecule [PECAM]), and von Willebrand factor (vWF), on seeded and nonseeded grafts. Coexpression with GFP was determined. For scanning electron microscopy, the vascular patch was fixed in 1% glutaraldehyde, 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), postfixed in 2% osmium tetroxide, dehydrated in acetone, critical point-dried using carbon dioxide, and coated by gold sputtering. Samples were examined with a GEMINI 982 LEO (Carl Zeiss Microimaging, Göttingen Standort Göttingen-Vertrieb, Deutschland) scanning electron microscope. All the surfaces were scanned, and 10 consecutive images representing the whole surface for each prosthesis were analyzed. Quantitative analysis was done using Optimas software (Imasys, Surennes, France) allowing localization of the endothelial surface using a histogram-based threshold. The endothelial covering area was expressed as a percentage of the total graft area. A part of prosthesis was also analyzed using JEOL 1010 (JEOL [Europe] SAS, Croissy-sur-Seine, France) transmission electron microscope in order to ensure the nature of cells after in vivo implantation. Ultrathin sections cut on Reichert Ultracut E (Leica Microsystems SAS, Rueil-Malmaison, Cedex, France), were contrasted with uranyl acetate and lead citrate before this last exploration.

**Statistical analysis.** Values between two groups were compared using the Mann-Whitney nonparametric test. Comparison between four groups was performed using a two-way analysis of variance. When an overall difference was found, a Holm-Sidak post hoc test of was done. All statistical analysis was performed using Minitab software (Minitab Inc, State College, Penn) and SigmaStat 3.0 (SysStat Software Inc, San Jose, Calif). P < .05 was considered statistically significant.

**RESULTS**

**Surgical graft implantation.** Twenty-four rats (n = 12 [first group] and n = 12 [second group]) were success- fully implanted with seeded polyurethane grafts and 12 with nonseeded polyurethane grafts (n = 12 [second group]). Aortic flow at the level of prosthesis was recorded by Doppler ultrasound before euthanasia. All rats implanted with a seeded graft (12 of 12 in group 1 and 6 of 12 in group 2) showed a normal flow velocity, whereas one-third of the rats (4 of 12) that received nonseeded grafts showed a decrease in aortic flow and exhibited inetroaortic obstructive thrombus formation on necropsy (Fig 1).

Mesenchymal stem cells in vivo proliferation and SMC differentiation was then evaluated.

**Smooth muscle protein expression.** Immunohistologic analysis of MSCs before seeding showed high positive α-SMA and GFP signals. No expression of SMMHC
or MLC2 was detected, contrary to what observed with mature SMCs (Fig 2).

Two weeks after in vivo implantation, a positive signal for α-SMA and GFP was observed on seeded grafts (n = 12; Fig 3). Sagittal view of the protheses showed that MSCs that were initially expended in a monolayer had proliferated in a thick multicellular layer mimicking the media of adjacent aortic vascular wall.

Seeded MSCs were thus collected and placed in growth medium. Of interest was that after 1 week of secondary

![Graph](image1.png)

**Fig 1.** A, Evaluation of endothelialization by scanning electron microscopy: (upper panel) original magnification ×100; (lower panel) original magnification ×1000. Endothelial cells (filled arrow) covered the seeded graft from the seventh day, although the non-seeded graft was essentially covered with collagen fibers (clear arrow), even after 14 days. B, Evaluation of percentage of endothelial surface on seeded graft (S) compared with nonseeded graft (NS) after 1 and 2 weeks in vivo. *Significantly different P < 0.05. C, Color flow Doppler shows homogenous flow velocity through aorta at the level of prosthesis (arrow).

![Graph](image2.png)

**Fig 2.** In vivo smooth muscle differentiation. A, Before implantation, undifferentiated mesenchymal stem cells (MSCs) expressed some smooth muscle proteins, including α-smooth muscle actin (SMA) but not others (desmin, smooth muscle myosin heavy chain [SMMHC], myosin light chain-2 [MLC2]) that are normally expressed by mature smooth muscle cells. The dark blue shows 4′-6-diamidino-2-phenylindole [DAPI] nuclei labeling. B, After in vivo implantation, MSCs seeded on graft were collected and placed in growth medium. They expressed smooth muscle proteins that were not detected before implantation (desmin, SMMHC, MLC2). C, Coexpression of green fluorescent protein (GFP) verified that the collected cells were the MSCs initially seeded on the graft. Secondary antibodies were Alexa (red) or phycoerythrin (green).
culture, they still had positive expressions for SMMHC, MLC2, and desmin, and also coexpressed a GFP signal, affirming that these collected cells were the seeded MSCs (Fig 2). Indeed, in vivo conditions have themselves induced smooth muscle maturation of MSCs. Expression of smooth muscle proteins-encoded genes (\(a\)-SMA, Desmin, SM-22a, MMHC-II) was still present before in vivo implantation. However SM-22a and MMHC-II enhanced expression after in vivo staying (Fig 4).

Functional phenotype characterization of mesenchymal stem cells. The current density was lower in undifferentiated MSCs compared with cells differentiated in vivo and to carotid and aortic SMCs but enhance after in vivo implantation. At \(+90\) mV, the current density was \(18.2 \pm 2.5 \, \text{pA/\mu F} \) (n = 7) for undifferentiated MSCs, \(45.8 \pm 10 \, \text{pA/\mu F} \) (n = 6) for in vivo differentiated cells \((P < .05)\), \(47 \pm 2 \, \text{pA/\mu F} \) (n = 6) for carotid cells \((P < .05)\), and \(38 \pm 6 \, \text{pA/\mu F} \) (n = 7) for aortic cells \((P < .05)\). Pretreatment with 4-aminopyridine (3mM) partially blocked the outward current on undifferentiated MSCs, whereas it had a lower effect on in vivo differentiated cells, suggesting that differentiation decreased 4-aminopyridine-sensitive channels.

Successive addition of iberiotoxin (100nM) induced a further decrease in current density. In undifferentiated MSCs, iberiotoxin inhibited the current by \(<10 \%\) for all the different voltage steps studied, whereas it significantly decreased in vivo–differentiated MSCs (Fig 5). Of interest was that a similar effect of iberiotoxin was observed in aortic SMCs, but it had a greater effect in carotid SMCs (Fig 5). This demonstrated that iberiotoxin mainly reduced the current in MSCs differentiated in vivo in a similar fashion to that in aortic SMCs.

Under controlled conditions (cells perfused with PSS), the resting cytoplasmic calcium concentrations ([Ca\(^{2+}\)]) were similar between undifferentiated and in vivo–differentiated MSCs. No changes were observed when the cells were perfused with PSS without calcium (Fig 6). In both types of MSCs, ATP (10\(\mu\)M), an inositol triphosphate dependant (IP3) agonist, induced a transient increase in [Ca\(^{2+}\)]. The amplitude of the signal and the percentage of the responding cells were similar. Similar responses were observed without an external solution in the bath. Thapsigargin (3 \(\mu\)M), a specific inhibitor of the sarcoplasmic reticulum ATPase calcium pump (SERCA), totally inhibited the [Ca\(^{2+}\)] increase in differentiated and undifferentiated cells. Caffeine (1 to 10mM), a ryanodine receptor activator, had no effects on each type of cell. Finally, contraction was never been observed with these collected cells, indicating that MSC had not archived to a functional smooth muscle cells phenotype.

Graft endothelialization. Immunohistologic analysis (Fig 6) showed large positive areas for CD31 and vWF on seeded grafts, whereas only small positive areas for CD31 and vWF were observed on nonseeded grafts (data not shown). Moreover, cells positive for CD31 and vWF existed on the unicellular layer, as observed in the physiologic wall. No coexpression of vWF with either GFP or anti-smooth muscle antibody was observed in the media layer, indicating that MSC did not give rise to these endothelial cells. Electron microscopy showed that these cells have a different morphology compared with the initial cells...
MSCs (Fig 7); however, the exact nature of these cells remained to be confirmed.

In nonseeded grafts, scanning electron microscopy showed incomplete endothelialization, even after 2 weeks. Collagen fibers were organized longitudinally (Fig 6), and when endothelialization was observed, it progressed from the periphery of the graft. In contrast, endothelialization on seeded grafts was observed in as early as 1 week, and full coverage of the seeded graft was observed after 2 weeks (Fig 7). The endothelial covering area was significantly increased in the seeded group compared with the nonseeded group, at 36% ± 7% vs 4% ± 2% (P < 0.05) after 1 week and 80% ± 6% vs 26% ± 5% (P < 0.05) after 2 weeks.

DISCUSSION

These data support, for the first time to our knowledge, that undifferentiated MSCs seeded on a graft are able to grow in vivo and to restore a thick multicellular layer mimicking mature vascular media. Although full SMC differentiation was not achieved, the MSCs exerted a potent and positive effect on vascular graft endothelialization and yielded a monolayer of endothelial cell restoration. This result underlies the potent, promising use of MSC-seeded prostheses in vascular surgery.

In the present study, we first observed that undifferentiated MSCs expressed smooth muscle–specific proteins after 2 weeks in vivo. Because we could not rule out the recruitment of local SMCs from the adjacent arterial wall, seeded MSCs were GFP-labeled. We observed that GFP-positive signals were coexpressed with smooth muscle–specific protein markers, proving that the SMCs detected and differentiated on grafts after implantation were the initially seeded MSCs. In our study, MSCs were only in contact with the recruited endothelial cells and no endothelial differentiation was observed. Wang et al21 showed in an in vitro model that cell-to-cell contact with mature corresponding cells was necessary to induce smooth muscle or cardiomyocyte differentiation, implicating the role of integrins in extracellular matrix interaction and cell adhesion mechanisms.22

Our results indicated that other factors such as pressure and stretch may contribute to MSC differentiation in vivo as observed in vitro.23,24 In this regard, other authors have emphasized the influence of pressure and stretch that could be mediated by the small Rho proteins family on vascular differentiation.23 Hegner et al25 focused on the involvement of mammalian target of rapamycin (mTOR) in the regulation of the smooth muscle phenotype of MSCs.
Other factors, such as periostin or growth factors, have also been listed. However, the precise mechanisms of in vivo vascular MSC differentiation remain poorly understood. Moreover, our data support the conclusion that MSCs did not reach functional contractile phenotype because electrophysiologic proprieties, calcium signaling, and contraction remained quite different than mature SMCs.

Second, we observed a very interesting impact of seeded MSCs on endothelial restoration. The impact of MSC seeding on thrombogenesis observed in our study.

Fig 5. Functional properties of undifferentiated and in vivo-differentiated mesenchymal stem cells (MSCs) compared with mature aortic and carotid smooth muscle cells (SMCs). A, Comparisons of (left panel) inhibitory effects of iberiotoxin (IBTX) and (right panel) 4-aminopyridine (4-AP) panel at +60 mV of holding potential. Results are expressed in percentage of inhibition of the potassium outward current. Smooth muscle from carotid (1) aorta (2) MSC (3), and MSC (4). *Significantly different \( P < 0.05 \). B, Typical example of differentiated cells in vivo (left image) before and (right image) after perfusion of 80mM potassium solution. In 30 cells, no contraction was observed. C, left panel, Typical trace showing adenosine triphosphate (ATP)-induced \( \text{Ca}^{2+} \) response in MSCs collected from the graft. ATP-induced \( \text{Ca}^{2+} \) response in undifferentiated MSCs and MSCs isolated from grafts (in vivo), in the presence (black columns) and in absence (white columns) of extracellular \( \text{Ca}^{2+} \). Error bars represent the standard deviation.

Fig 6. Confocal microscopy analysis of a graft seeded with mesenchymal stem cells after in vivo implantation shows expression of endothelial markers. A, Before in vivo implantation, no endothelial marker expression was attested. B, After in vivo implantation, the frontal view showed a CD31-positive area on the prosthesis that looked like the adjacent aortic vascular wall (arrow, anastomosis). C and D, Sagittal view showed endothelium-specific antigen (C) CD31 and (D) von Willebrand factor, which were observed only in the first layer in contact with the artery lumen.
must be confirmed in human studies. A previous study used a scaffold containing MSCs for embedding in the carotid and showed potential for preventing thrombosis and remodeling. In vivo endothelial differentiation of MSCs has been evoked after myocardial transplantation or venous implantation because these cells coexpress endothelial protein expression.

In the present study, we obtained several lines of evidence to rule out such endothelial differentiation of MSCs. Only the superficial layers of MSC-seeded grafts were positive for CD31 or vWF, whereas the media layer was systematically negative for these markers. What is the origin of these endothelial cells? Although endothelial cells could arise from endothelium adjacent to the prosthesis, circulating endothelial progenitors could also contribute to prosthesis endothelialization. Indeed, it is known that MSCs induce angiogenesis in myocardium after transplantation and that their paracrine properties, including production of several factors, such as vascular endothelial growth factor, fibroblast growth factor-β and stromal cell-derived factor-1, are yet well-identified. Previous studies have shown that MSCs can promote in vitro endothelial cell migration. However, the interactions between MSCs and endothelial progenitors are unknown, and the precise nature of endothelial cells covering the MSCs seeded on grafts remains to be investigated. Finally, the use of undifferentiated MSCs for endothelial restoration seems to be a very promising tool because graft recovery of >90% of the surface was obtained after 1 week of culture that allows performing surgical procedures with a short delay of time.

CONCLUSION

In vivo conditions induced incomplete smooth muscle differentiation of MSCs and no endothelial differentiation. Despite this, the monolayer of undifferentiated MSCs gave rise to a multilayer one with an endothelium-like lumen border. The monitoring of endothelium restoration using a MSC-seeded prosthesis could be a promising therapeutic tool for vascular surgery in humans.

We thank Georges Kahmis for his technical assistance.

AUTHOR CONTRIBUTIONS

Conception and design: VE, PL
Analysis and interpretation: VE, JMH, DA
Data collection: AM, BM, JMH, PB
Writing the article: VE, PB, AB
Critical revision of the article: JD
Final approval of the article: AM, JMH, BM, PL, PB, JD
Statistical analysis: AB
Obtained funding: VE
Overall responsibility: VE

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