ORIGINAL CONTRIBUTION

# Potential of Human Umbilical Cord Matrix and Rabbit Bone Marrow–Derived Mesenchymal Stem Cells in Repair of Surgically Incised Rabbit External Anal Sphincter

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**PURPOSE:** Anal sphincter defects and fecal incontinence are complicated surgical problems. We investigated the ability of human umbilical cord matrix (hUCM) and rabbit bone marrow (rBM) stem cells to improve anal sphincter incontinence due to induced sphincter defects without surgical repair.

**METHODS:** We harvested hUCM cells from human Wharton's jelly and rBM stem cells from rabbit femurs and tibias. To induce sphincter defects, we made an incision in the external anal sphincter. Rabbits were randomly allocated to 5 groups to receive either no intervention (n = 3) or injections of $10^4$ hUCM cells in medium (10 $\mu$L RPMI-1640), rBM cells in medium, medium only, or normal saline (n = 7 per group), 2 weeks after sphincterotomy. Transplanted cells were tracked in the injured sphincters by prelabeling with bromodeoxyuridine. Electromyography was performed before and 2 weeks after the external anal sphincterotomy, and 2 weeks after cell transplantation. We also evaluated the proliferation and differentiation of injected cells with histopathologic techniques.

**RESULTS:** Electromyography showed significant improvement in sphincter function 2 weeks after local injection of rBM stem cells compared with pretreatment values and controls. Moderate, nonsignificant improvement was observed with hUCM cell injection. Cells with incorporated bromodeoxyuridine were detected at the site of injury after transplantation of hUCM and rBM. Histopathologic evaluation showed normal or muscle-dominant sphincter structure in all animals receiving rBM and fibrous-dominant sphincter structure in most animals receiving hUCM.

**CONCLUSIONS:** Stem cell injection at the site of injury can enhance contractile function of the anal sphincter without surgical repair. Transplantation of stem cells, particularly bone marrow mesenchymal cells, may provide an effective tool for treating anal sphincter injuries in humans.

**KEY WORDS:** Anal sphincter; Rabbit; Bone marrow–derived mesenchymal stem cells; Human umbilical cord matrix cells; External anal sphincterotomy; Electromyography.

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Fecal incontinence, which can range from a daily loss of all stools to occasional soiling, is a gastrointestinal problem that significantly impairs quality of life.[1] Although estimates of prevalence rates vary, a recent consensus statement estimated the rate of fecal incontinence in noninstitutionalized persons at 6% in women younger than 40 years, 15% in women older than 40, and 6 to 10% in men.[2] Aging is a major risk factor for fecal incontinence, and older people living in residential homes are particularly affected, with at least twice-monthly episodes reported in more than 16% of residents and at least weekly episodes in 10%.[3] It is not known whether aging directly affects sphincter function or aggravates the effect of an anal sphincter tear, but in long-term studies in women, only 6% of cases were due to an anal sphincter tear.[4] In most patients, the cause of incontinence is likely to be a cumulative, multifactorial process.[5] Nonsurgical options for treatment include medications to slow transit or increase stool consistency, special diets, habit training, and biofeedback with sphincter exercise, but these approaches appear to achieve complete continence in no more than approximately 50 to 60% of patients.[6] Surgical options for correction of fecal incontinence, which include procedures such as direct sphincter repair, artificial sphincter implantation, and colostomy diversion, also achieve varying rates of success.[7]

The most recent suggested procedure for treatment of fecal incontinence is regenerative medicine. The ability of bone marrow (BM) stem cells to form muscle *in vitro* and *in vivo* has been previously demonstrated,[8] and BM cells have been used as a source of cell therapy in animal models.[9–12] Umbilical cord matrix (UCM) cells are embryonic derivatives of Wharton's jelly which are easily acquired and propagated in cell culture laboratories. These cells have been recently introduced as a promising source of stem cells, and their capacity to differentiate into chondrocyte, neurons, glia, and cardiac muscle has been demonstrated in some species.[12–15] A recent study in rats showed the ability of a combination of surgical repair and injection of bone marrow stem cells to correct fecal incontinence.[9]

The present study was designed to find out whether stem cell therapy may improve anal sphincter incontinence without surgical repair. We evaluated the potential of rabbit bone marrow (rBM) and human umbilical cord matrix (hUCM) stem cells in repair of fecal incontinence resulting from surgical division of the rabbit external anal sphincter (EAS sphincterotomy).

## MATERIALS AND METHODS

This study was performed in accordance with the guidelines for the care and use of laboratory animals established by the local ethics committee at Kerman University of Medical Sciences, Kerman, Iran.

### Animals

Thirty-five male white New Zealand rabbits (weight range 2.5–3.5 kg) provided from Razi Institute (Karaj, Iran) were assigned to the experiments. Four of these were used for bone marrow stem cell collection. The remaining animals were kept in an animal room with a 12-hour-day/night cycle and free access to water and chewing rodent food.

### Experimental Design

All animals used for the comparative study underwent clinical evaluation for anal sphincter performance and EAS sphincterotomy. Animals were randomly allocated to 5 groups to receive the following treatment: 1) viable hUCM cells in medium; 2) viable rBM cells in medium; 3) medium only; 4) normal saline; or 5) no intervention. All study preparations were injected at the site of injury. Groups 1 through 4 contained 7 animals each, and the treatment-free control group contained 3 animals.

In all groups, clinical examination and electromyography (EMG) were performed before the sphincterotomy, 2 weeks after the sphincterotomy but before stem cell injection, and 2 weeks after stem cell injection. After the final EMG, animals were killed and the EAS was removed for immunohistochemical and histopathologic evaluations.

### Chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO) unless otherwise stated.

### rBM Cell Culture

rBM cells were collected after the rabbit femur and tibia marrow cavity were extensively flushed out with heparinized Hank's balanced salt solution (HBSS). After aseptic marrow draining, the collected suspension was centrifuged over 50% Pure-Sperm (Nidacon, Göteborg, Sweden; a density gradient solution used for sperm preparation in assisted reproductive technology procedures) in HBSS at $350 \times g$ for 20 minutes. The supernatant fraction was collected aseptically and centrifuged at $500 \times g$ for 10 minutes after washing with HBSS. Pelleted cells were cultured in 25-$cm^2$ Falcon cell culture flasks (Becton Dickinson and Company, Franklin Lakes, NJ) in RPMI-1640 supplemented with 20% fetal bovine serum (FBS; Gibco, Grand Island, NY), 100 IU/ml penicillin, and 60 $\mu$g/ml streptomycin. Flasks were incubated at 37°C in a humidified atmosphere containing 5% $CO_2$ in the air. After overnight incubation, the floating cells were discarded and the attached cells were harvested in the same medium. Once the adherent fractions had reached >80% confluence, the cells were trypsinized with 0.5 g/l trypsin and 0.2 g/l EDTA in phosphate buffered saline (PBS) for cell transplantation or cryopreserved in liquid nitrogen for further use.

**TABLE 1.** Summary of treatments received by each study group

| *Study group* | *N* | *EAS sphincterotomy* | *hUCM ($10^4$ cells)* | *rBM ($10^4$ cells)* | *Culture medium* | *Cyclosporin A* | *PBS* |
|---|---|---|---|---|---|---|---|
| hUCM | 7 | + | + | − | + | + | − |
| rBM | 7 | + | − | + | + | + | − |
| Medium | 7 | + | − | − | + | − | − |
| Saline | 7 | + | − | − | − | − | + |
| No treatment | 3 | + | − | − | − | − | − |

EAS = external anal sphincter; hUCM = human umbilical cord matrix; PBS = phosphate buffered saline; rBM = rabbit bone marrow; + = treatment administered; − = treatment not administered.

### *hUCM Cell Culture*

The procedures for collecting human umbilical cords were approved by the local ethics committee at Kerman University of Medical Sciences, Kerman, Iran. Human umbilical cords were collected aseptically from cesarean section normal live births after written informed consent was obtained from the mother. Umbilical cords were transferred in HBSS with 100 IU penicillin G, 60 $\mu$g/ml streptomycin, and 50 $\mu$g/ml amphotericin B to the laboratory equipped with cell culture facilities. In the cell laboratory, the umbilical cords were carefully dissected free of the amniotic membrane and the cord vessels with fine forceps and a pair of scissors. The Wharton's jelly was washed several times with PBS, minced into small (5- to 6-mm) pieces, and put in 3-cm Petri dishes; then 1 mL Dulbecco's modified Eagle medium F-12 (DMEM/F12) supplemented with 20% FBS, 100 IU/ml penicillin, and 60 $\mu$g/ml streptomycin was added slowly to each plate. Plates were kept in a 37°C humidified incubator with 5% $CO_2$ in the air. After 24 hours, 2 mL of the same medium was added to each Petri dish. The explants were detached from the plates and discarded after 5 to 6 days of cultivation. When the propagated cells reached confluence of 80 to 90%, the cells were passaged after treatment with 0.5 g/l trypsin and 0.2 g/l EDTA in PBS and subcultured under the previous condition. Cells at earlier passages (2–6) were used for further experiments.

### *Labeling of Transplanted Cells*

For tracking the transplanted cells in the injured sphincters, the cells were prelabeled with bromodeoxyuridine (BrdU; Chemicon-Millipore, Temecula, CA) before the transplantation was carried out.[16] Briefly, each type of stem cell (rBM and hUCM) was incubated with 10 $\mu$M of BrdU in the culture medium for 48 hours in a humidified 37°C incubator with 5% $CO_2$ in the air. The efficacy of BrdU incorporation was assessed in a pilot study by staining with a BrdU detection kit that used a biotinylated monoclonal antibody to BrdU (BrdU Immunohistochemistry Kit, Chemicon-Millipore).

### *Surgical Procedures and Injection of Study Preparations*

Rabbits were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine, and then fixed in the lithotomy position. In each animal, a sphincterotomy was performed in the right lateral portion of the EAS.

Two weeks after the EAS sphincterotomy, either a stem cell or a control preparation was injected at the site of the EAS sphincterotomy scar with a 27-gauge needle attached to a Hamilton syringe. The treatments received by each group are summarized in Table 1. The stem cell groups received $10^4$ cells of either hUCM or rBM suspended in 10 $\mu$L of RPMI-1640. Control animals in the medium group received 10 $\mu$L of RPMI-1640, and those in the saline group received 10 $\mu$L of PBS. To suppress the probable immune response to the stem cell preparations, rabbits in the hUCM and rBM groups received an intramuscular injection of 10 mg/kg cyclosporine A (Novartis, Täby, Sweden) once per day, for 2 weeks.

### *Clinical Evaluation*

Clinical examination of all animals was performed before the sphincterotomy, 2 weeks after sphincterotomy (just before injection of the study preparation), and 2 weeks after administration of the study preparation. Any sign of incontinence was noted, including flaccid atonic sphincter and open anal orifice with loose fecal consistency.

### *Electromyography of the Sphincter*

Electromyography was carried out before the sphincterotomy (pre–EAS division), 2 weeks after sphincterotomy just before injection of the study preparation (post–EAS division), and 2 weeks after administration of the study preparation (posttreatment). The Medelec-Synergy EMG monitoring system (Cardinal Health NeuroCare, Madison, WI) and the Oxford recording program designed for this device were used. The procedure was carried out for all animals in each group as follows: After shaving the thigh and establishing a ground connection, we inserted a Medelec ELITE disposable concentric 26-gauge needle electrode (Cardinal Health), which had a 0.46-mm diameter, 25-mm length, and 0.07-$mm^2$ recording area, at the 9 o'clock position of the anal canal. The needle was inserted through the perianal skin adjacent to the mucocutaneous junction; the tip of the electrode entered perpendicular to the skin close to the anal orifice (0.2 cm from the anal ring). The device was set at a 5- to 10-millisecond/cm sweep

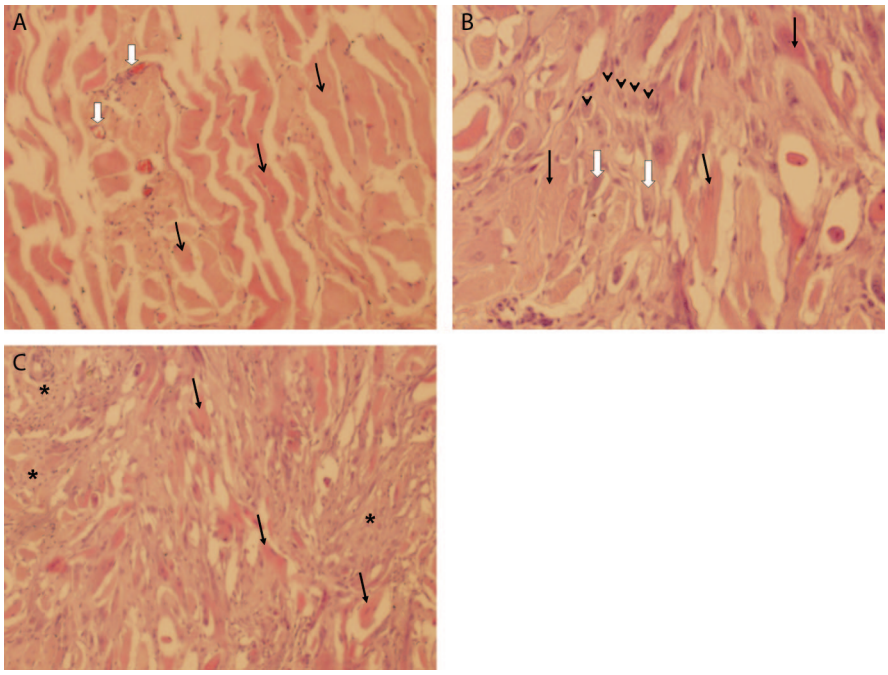


**FIGURE 1.** Histologic classification of sphincters after cell transplantation. A. Normal sphincter with normal muscle fibers (black arrow) and some vessels (white arrow). B. Muscle-dominant sphincter (example from rBM group). Black arrows show mature muscle fibers, white arrows show maturing muscle fibers, and arrow heads show active mesenchymal cells. C. Fibrous-tissue–dominant sphincter. Asterisks show areas of connective tissue; arrows show normal or degenerating muscle fibers. Original magnification in A and B, ×200; in C, ×100.

speed and 100 to 200 $\mu$V of gain; motor units per 20 seconds were recorded during anal functional sphincter activity (contraction).

### *Immunohistochemistry and Histopathology*

Two weeks after administration of the study preparations (after the final EMG recording), animals were killed by intracardiac injection of 10% potassium chloride in distilled water. The EAS was excised carefully, and the site of the EAS sphincterotomy (the site where the cells were injected) was marked using a simple suture. The specimens were fixed in 10% formaldehyde in PBS for 24 hours. Tissues were prepared with routine histologic procedures and 5-$\mu$m thick sections were arranged and stained with hematoxylin and eosin.

***Identification of Engrafted Cells.*** Five-$\mu$m thick slices were deparaffinized and rehydrated, and the DNA was denaturized using 2N HCl for 30 minutes at room temperature. Samples were rinsed in PBS, incubated for 30 minutes at 37°C with trypsin, washed with PBS, and incubated for 10 minutes in 5% hydrogen peroxide to block endogenous peroxidase activity. Samples were then washed with tris buffer (pH = 7), and monoclonal anti-BrdU (BrdU Immunohistochemistry Kit, Chemicon-Millipore) was applied to each slide for 45 minutes in a humidified environment at 37°C. Slides were washed 2 times with tris buffer, and goat anti-mouse IgG labeled with peroxidase was added to each slide and incubated at 37°C for 30 minutes. After the slides were washed with PBS, diaminobenzidine (DAB) was added to each slide and incubated at room temperature for 30 minutes. After being washed with distilled water, the slides were counterstained with hematoxylin and prepared for microscopic observation. Slides were carefully evaluated with a light microscope equipped with a digital camera (Olympus, Japan).

***Evaluation of Tissue Structure.*** The prepared slides were carefully reviewed by a pathologist who was blind to the arrangements of intervention. The pathologist evaluated the presence of newly formed striated muscles at the site of cell transplantation and reported the results in 3 categories as follows: 1) normal structural sphincter in which hypertrophied regular sphincter muscle was observed; 2) muscle-dominant sphincter containing a mixture of muscle and fibrous tissue, with muscular structures predominant; and 3) fibrous tissue–dominant sphincter in which fibrous structures were predominant (Fig. 1).

### *Statistical Analysis*

Data from EMG recordings are presented as mean ± standard error of the mean (SEM) for each group. The efficacy of EAS sphincterotomy was tested by comparing pre–EAS division and post–EAS division EMG data within groups by use of a paired-sample *t* test or a nonparametric equivalent test, depending on whether normality could be assumed. Post–stem cell injection EMG data and histopathologic results in different groups were analyzed by use of the

**TABLE 2.** Results of electromyography before and after sphincterotomy and after treatment with stem cells or a control preparation

| Study group | n | Pre–EAS division | Post–EAS division | Posttreatment |
|---|---|---|---|---|
| hUCM | 5 | 37.25 ± 6.57 | 0.06 ± 0.60 | 11.25 ± 7.16 |
| rBM | 5 | 24.64 ± 2.79 | 0.54 ± 0.54[a] | 13.59 ± 2.97[b] |
| Medium | 6 | 35.28 ± 3.87 | 0.25 ± 0.25 | 4.75 ± 3.04[c] |
| Normal saline | 6 | 33.33 ± 2.47 | 0.40 ± 0.40 | 1.10 ± 1.10[d] |
| No treatment | 3 | 21.33 ± 7.72 | 0 | 0[e] |

rBM = rabbit bone marrow; EAS = external anal sphincter; hUCM = human umbilical cord matrix. • Data are mean spikes/second ± standard error of the mean before EAS sphincterotomy (pre–EAS division), 2 weeks after EAS sphincterotomy (post–EAS division), and 2 weeks after injection of study preparation (posttreatment). • Each group is labeled with a superscript digit denoting significant between-group comparisons as follows: a *vs.* b, $P < 0.01$; b *vs.* c, $P < 0.05$; b *vs.* d, $P < 0.01$; and b *vs.* e, $P < 0.001$.

Kruskal-Wallis test followed by the Mann-Whitney *U* test for evaluation of significance of differences between groups. Data analysis was performed using SPSS for Windows software, Version 11.5 (SPSS, Chicago, IL). *P* values <0.05 were considered significant.

## RESULTS

In this study, 25 rabbits withstood the surgical and treatment procedures; 6 rabbits died, 2 in each stem cell group, one in the medium group, and one in the saline group.

### Results of Clinical Examination

No sign of fecal incontinence was detected in any of the animals before surgical intervention. Two weeks after EAS sphincterotomy all the excised animals had a flaccid sphincter with an open anal orifice, and most had loose fecal consistency. Two weeks after cell transplantation (at the time of the EMG recording), the EAS was judged to be competent in 4 animals in the rBM group and in 2 animals in the hUCM group, and moderately functional in 2 animals in the group that received medium alone.

### Results of Electromyography

Pre–EAS division EMG data were analyzed to ascertain whether differences existed among groups that would indicate problems with randomization. No significant difference among treatment groups was detected (Table 2). As shown in Figure 2, electromyography 2 weeks after EAS sphincterotomy (just before administration of the study preparations) demonstrated a significant overall decrease in mean spikes per second compared with pre–EAS division values ($P < 0.001$), indicating that the sphincterotomy was successful. No significant differences were observed among groups after EAS sphincterotomy (post–EAS division) (Table 2).

As also shown in Table 2, the mean number of spikes per second increased significantly after administration of rBM ($P < 0.01$). In addition, posttreatment EMG values were significantly higher in the rBM group than in the 3 control groups. In the hUCM group, the number of spikes per second increased after treatment, but not significantly, and posttreatment differences between the hUCM group and control groups were not significant. No significant improvement in EMG values was observed after local injection of medium or normal saline at the site of injury. EMG traces from a normal rabbit sphincter and representative examples of the study treatments are shown in Figure 3.

### Immunohistochemistry

On slides stained with the BrdU detection kit, the nuclei of cells with incorporated BrdU were colored brown, whereas other cells had a bluish hematoxylin nuclear stain (Fig. 4A). The efficacy of BrdU incorporation was estimated in the pilot study to be approximately 50 to 60%.

BrdU-positive cells were detected at the site of injection both in animals receiving rBM (Fig. 4B) and in those receiving hUCM (Fig. 4C).

### Histopathologic Evaluation of Sphincter Structure

Results of the histopathologic evaluation of sphincter structure are shown in Table 3. All animals that received rBM cells had normal or muscle-dominant sphincter structure, whereas most animals that received hUCM had fibrous-dominant sphincter structure. The difference between groups was not statistically significant ($P = 0.26$). However, the difference between the results of the rBM group and the normal saline group was nearly significant ($P = 0.054$).

## DISCUSSION

The present study investigated the efficacy of transplantation of 2 types of stem cells in repairing complete EAS

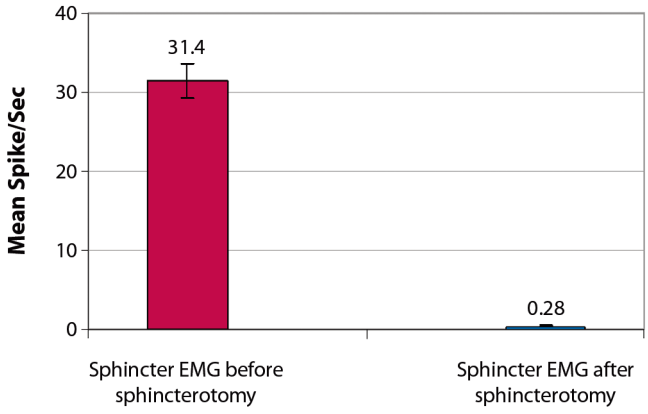


**FIGURE 2.** Results of electromyography (EMG) before and 2 weeks after sphincterotomy of the external anal sphincter (EAS), showing a significant decrease in mean spikes/second (± standard error of the mean) ($P < 0.001$).

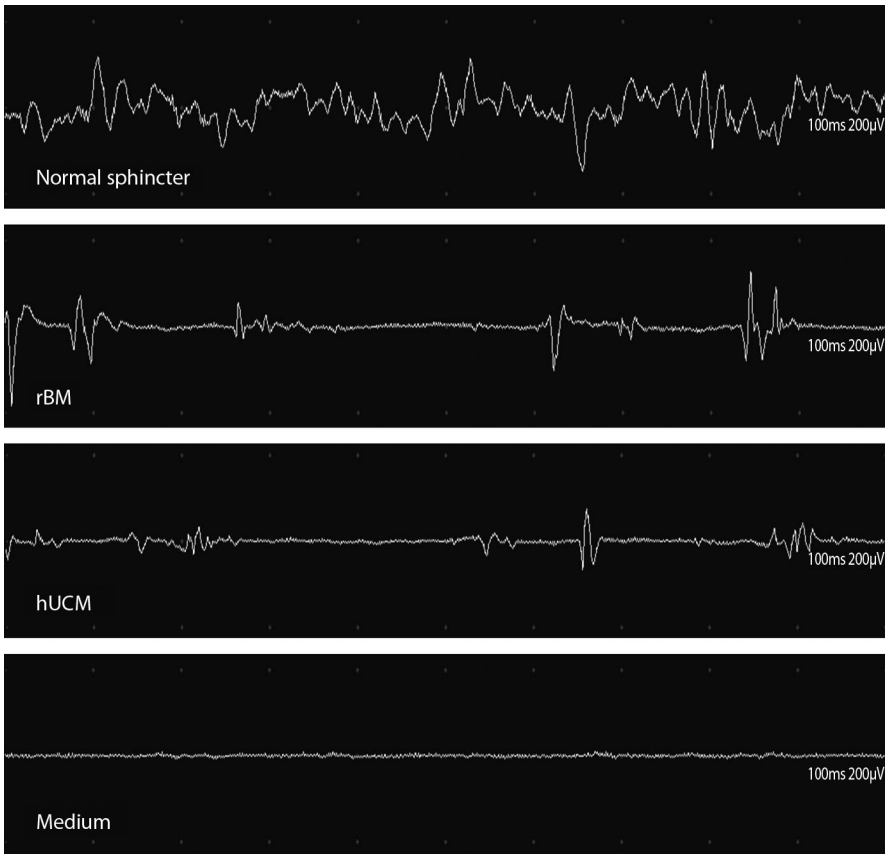


**FIGURE 3.** Electromyography traces in various groups. EMG traces from no treatment, normal saline and medium were nearly identical; therefore, a trace from medium group is presented.

defects in rabbits without surgical intervention. rBM cell transplantation significantly improved defects in sphincter anatomy and physiology caused by sphincterotomy. A recent study has demonstrated the efficacy of a combination of primary surgical repair and bone marrow stem cell injection for treatment of surgically injured anal sphincters in rats.[9] However, to our knowledge, stem cell injection has not previously been used to treat anal sphincter injuries without surgical repair. In previous studies, injection of both autologous myoblasts and fibroblasts has been shown to have a positive effect on urinary incontinence in women.[17] Similar results in human male urethral sphincter defects following prostatectomy were reported by the same group after they used a combination of myoblasts and fibroblasts.[18]

In our study, electromyographic recordings showed a significant improvement in sphincter muscle function in rabbits that received rBM cells, and a nonsignificant, relative improvement in rabbits that received hUCM cells. Our clinical observations confirmed this finding, as we observed competent sphincters in animals that showed improvement in EMG values after receiving stem cells, and atonic nonfunctional sphincters in those whose EMG values did not improve after intervention.

Four of the 5 rabbits in the rBM group had higher EMG values than any of the control rabbits receiving culture medium, and the sphincter of only one rabbit treated with rBM was flaccid after cell therapy. Greater variation was found in the hUCM group: Although fewer animals in this group had improved anal sphincter tone after treatment, 2 of the 5 showed greater improvement in results of EMG than any animals that received rBM (data not shown). We do not have a definitive explanation for such uneven results. Problems in cell preparation, the technique of cell transplantation, or to a lesser extent the EMG recording may be the source of the variation.

The plasticity of muscular tissue following injury has recently been demonstrated.[10] In our study, detection of the nuclear cell marker BrdU in cells at the site of injury, as well as our histopathologic findings of new muscle fibers in the cell-transplanted area (Fig. 4B) after local injection of stem cells, suggest that transplanted stem cells did indeed contribute to the formation of new muscular tissue at the site of injury. However, we did not use myogenic cell markers in conjunction with anti-BrdU staining to confirm the origin of such newly formed muscle fibers. The exact contribution and differentiation of transplanted stem cells into muscular tissue should be explored by double labeling

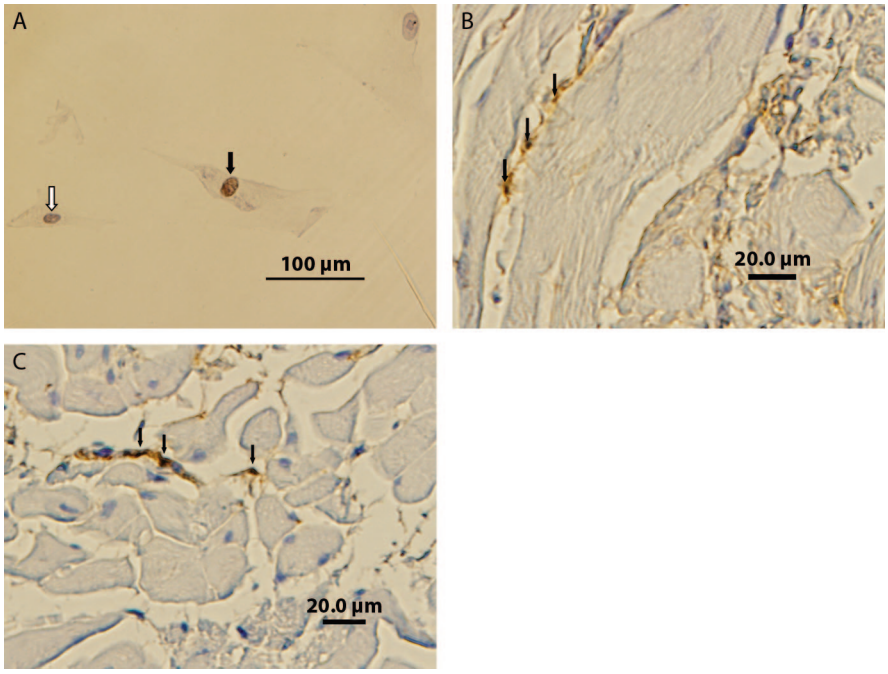


**FIGURE 4.** A. The nucleus of a cell with incorporated BrdU is colored dark brown (black arrow), and the nucleus of a cell without incorporated BrdU is colored pale blue (white arrow). B and C show cells with incorporated BrdU (black arrows) between sphincter cells of rabbits treated with hUCM (B) or rBM (C).

of engrafted cells with a cell proliferation marker and a specific myocyte marker.

Bone marrow stem cells may differentiate into various cell types, including chondrocytes, osteocytes, neuron-like cells, and myocytes, *in vitro* as well as *in vivo*.[8] The milieu after transplantation appears to have an effect on differentiation of transplanted cells; for example, when human bone marrow–derived mesenchymal stem cells are transplanted into a damaged heart, myogenic factors such as TNF, IL6, and CRP produced from host cardiomyocytes may influence the differentiation of the transplanted cells into mature contracting cardiomyocites.[19–22] Although our observation of active mesenchymal cells and maturing muscle fibers in cell transplanted areas (Fig. 1B) suggests that a similar mechanism may be involved in sphincter improvement following cell therapy, the precise mechanism of differentiation of stem cells into muscle tissues remains to be explored.

**TABLE 3.** Distribution of various sphincter structures found on histopathologic examination 2 weeks after administration of study preparations

| | | *Sphincter structure* | | |
|---|---|---|---|---|
| *Study group* | *n* | *Normal* | *Muscle-dominant* | *Fibrous-dominant* |
| hUCM | 4* | 1 | 0 | 3 |
| rBM | 5 | 4 | 1 | 0 |
| Medium | 6 | 2 | 1 | 3 |
| Saline | 5* | 1 | 2 | 2 |
| No treatment | 3 | 2 | 0 | 1 |

hUCM = human umbilical cord matrix; rBM = rabbit bone marrow.
*The results of one sample in the Saline group and one sample in the hUCM group were excluded because of poor quality of the processed tissues.

hUCM and rBM cells are known to secrete many growth factors which contribute to cell propagation and differentiation (refer to Powell et al. 1999[23] for a review). hUCM cells have been described as having the characteristics of myofibroblasts,[12] which play a role in healing by producing a wide range of cytokines, growth factors, chemokines, and inflammatory mediators and can produce both insulin-like growth factors (IGF) I and II. IGF-I has been shown to be a powerful mitogenic factor for myocyte cells and has been previously suggested for the treatment of progressive muscular dystrophy.[24] Some authors have discussed paracrine effects of transplanted stem cells on host tissue stem cells to proliferate and differentiate inside the injured area.[25,26] Although we could not define the exact mechanism by which stem cells improved rabbit sphincter activity in our study, either a direct contribution of transplanted stem cells to repair of the injured area,[15,16] host stem cell mobilization and activation in response to paracrine factors secreted by transplanted cells,[25,26] or a combination of these mechanisms may have contributed to our results.

Umbilical cord matrix cells originate early in embryogenesis (around day 12 of gestation) from extra-embryonic

mesoderm.[27] Due to their embryonic nature, UCM cells may not induce immune responses or produce tumors in the host.[15] It has been shown that xenotransplantation of porcine UCM cells into rat brain had not triggered an immune response after 2 to 6 weeks of follow-up.[28] This finding was supported by data on transplantation of hUCM cells into the brain of hemiparkinsonian rats.[15] However, we were not certain whether the hUCM cells used in our study would elicit an immune response in the host animals. Therefore, we suppressed the immune system of recipient rabbits in both experimental groups by daily injection of cyclosporine A. Further studies should be conducted to define the extent of antigenic properties of hUCM cells with confidence. If their nonimmunogenic properties can be confirmed, hUCM cells may potentially serve as a good source of stem cells for repair of damaged tissues in humans.

Scar formation at the site of injury is a common phenomenon leading to weakness of the sphincter and can be a problem in clinical practice, because surgery to repair the sphincter is often delayed. In the present study, we injected stem cells at the site of injury 2 weeks after EAS sphincterotomy, at a time when fibrosis had previously occurred and resulted in scar formation. Our data suggest that administration of rBM cells, and to a lesser extent hUCM cells, may aid repair of anal sphincter defects even after a delayed injury.

The present study demonstrated promising effects of transplanted nonembryonic stem cells for repair of anal sphincter defects without surgical intervention in an animal model. One limitation of our study is that we did not assess the effective contraction of the sphincter by manometer. Further studies should evaluate other aspects of transplanting such cells; for example, the optimal number of stem cells, the exact method of injection, the most appropriate time of injection in the therapeutic procedure, the advantages and disadvantages of autologous stem cell transplantation, and the effects of combining cell transplantation with administration of growth factors. A controlled clinical trial will be needed to illuminate the long-term efficacy and safety of stem cell therapy for treatment of fecal incontinence in humans.

## CONCLUSION

Our study suggests that bone marrow mesenchymal cells, and to a lesser extent human umbilical cord matrix cells, may be effective in treating anal sphincter injuries in future clinical applications of stem cell therapy. Although further studies are required to confirm and increase our knowledge before this method can be used in the clinical setting, such cells may be able to repair anal sphincter defects and contribute to normalizing the physiology of injured sphincters.

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