Dental Apical Papilla as Therapy for Spinal Cord Injury


Abstract
Stem cells of the apical papilla (SCAP) represent great promise regarding treatment of neural tissue damage, such as spinal cord injury (SCI). They derive from the neural crest, express numerous neurogenic markers, and mediate neurite outgrowth and axonal targeting. The goal of the present work was to investigate for the first time their potential to promote motor recovery after SCI in a rat hemisection model when delivered in their original stem cell niche—that is, by transplantation of the human apical papilla tissue itself into the lesion. Control groups consisted of animals subjected to laminectomy only (shams) and to lesion either untreated or injected with a fibrin hydrogel with or without human SCAP. Basso-Beattie-Bresnahan locomotor scores at 1 and 3 d postsurgery confirmed early functional decline in all SCI groups. This significant impairment was reversed, as seen in CatWalk analyses, after transplantation of apical papilla into the injured spinal cord wound, whereas the other groups demonstrated persistent functional impairment. Moreover, tactile allodynia did not develop as an unwanted side effect in any of the groups, even though the SCAP hydrogel group showed higher expression of the microglial marker Iba-1, which has been frequently associated with allodynia. Notably, the apical papilla transplant group presented with reduced Iba-1 expression level. Masson trichrome and human mitochondria staining showed the preservation of the apical papilla integrity and the presence of numerous human cells, while human cells could no longer be detected in the SCAP hydrogel group at the 6-wk postsurgery time point. Altogether, our data suggest that the transplantation of a human apical papilla at the lesion site improves gait in spinally injured rats and reduces glial reactivity. It also underlines the potential interest for the application of delivering SCAP in their original niche, as compared with use of a fibrin hydrogel.

Keywords: dental stem cells, fibrin hydrogel, spinal cord hemisection, pain, regenerative medicine, central nervous system

Introduction
One of the objectives when developing therapies for spinal cord repair is the stimulation of functional recovery. In that context, dental stem cells are an attractive source of stem cells for cell-based therapies due to their accessibility, their neural crest origin, their great proliferation rate, and their potential for autologous transplantation (Giuliani et al. 2013). Human dental stem cells display superior neural stem cell properties than do bone marrow–derived mesenchymal stem cells (Huang et al. 2009; Karaouz et al. 2011). Different populations of dental stem cells have been identified (Huang et al. 2009). Dental pulp stem cells secrete neurotrophic factors and promote survival of embryonic dopaminergic neurons (Nosrat et al. 2004). Stem cells of the apical papilla (SCAP) were identified as the main source of undifferentiated cells involved in the process of root development (Ruparel et al. 2013) and were shown to possess a higher proliferation potential than that of dental pulp stem cells (Martens et al. 2013). Importantly, even in an undifferentiated state, SCAP have been shown to express a variety of neuronal markers (Huang et al. 2008; Huang et al. 2009) as well as to produce and secrete a range of growth factors (Arthur et al. 2009; Martens et al. 2013).

Transplantation of dental stem cells for spinal cord injury (SCI) repair has already been shown to induce functional recovery. Indeed, Sakai et al. (2012) demonstrated that SHED (stem cells from human exfoliated deciduous teeth) were able to differentiate toward an oligodendrocyte phenotype in vivo in an SCI model and to promote recovery by altering macrophage polarity (Matsubara et al. 2015). However, access to SHED could be limited and challenging, and the potential for autograft would be reduced.

Keywords:
- Dental stem cells
- Fibrin hydrogel
- Spinal cord hemisection
- Pain
- Regenerative medicine
- Central nervous system

Authors contributing equally to the work.

A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

Corresponding Author:
A. des Rieux, Université Catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Brussels, Belgium

Email: anne.desrieux@uclouvain.be
To increase cell viability and to ensure their concentration at the site of delivery, transplantation of cells within their own extracellular matrix (Li et al. 2003) or a polymeric scaffold could be used (Fühmann et al. 2011). However, isolating cells from their natural niche and their culture on plastic could modify their properties (Ruparel et al. 2013). Therefore, the delivery of stem cells maintained in their original niche is of potential interest. Specifically, spinal lesion cavities have been implanted with solid tissues, such as fetal embryonic tissue (Coumans et al. 2001) and peripheral nerves (Cheng et al. 1996). To our knowledge, implantation of a whole human apical papilla in a spinal cord lesion has never been reported.

Therefore, the main objective of this study was to test the null hypothesis that the transplantation of a whole apical papilla into a spinal cord hemisection at T10 in a rat model affected 1) motor recovery (evaluated by CatWalk) and 2) allodynia (evaluated by Von Frey filaments), as compared with various control groups.

**Material and Methods**

**Apical Papilla Extraction**

Wisdom teeth were collected from 6 healthy individuals aged between 16 and 18 y. An informed consent was obtained from all donors (UCL/2012/14JUN/283). Apical papilla tissue was loosely attached and could be easily separated from the apex of the developing root with a pair of tweezers (Huang et al. 2008).

**Dental SCAP**

Previously characterized human SCAP between passages 3 and 5 were used (RP89 cells; Ruparel et al. 2013). Mesenchymal stem cell markers (CD90, CD73, CD105) are homogenously coexpressed by RP89 cells (97% of the population; Ruparel et al. 2013), and RP89 cells are multipotent (Vanacker et al. 2014).

**Fibrin Hydrogel Formulation**

Fibrinogen and thrombin were reconstituted according to the supplier instructions (Baxter Innovations GmbH, Vienna, Austria) to obtain 100 mg/mL of fibrinogen and 500 IU/mL of thrombin solutions (Germain et al. 2015). Diluted solution of fibrinogen (50 mg/mL) and thrombin (10 IU/mL) were prepared, and fibrin hydrogels were formed by mixing equal volumes of fibrinogen and thrombin-diluted solutions.

**Spinal Cord Hemisection**

Animal experiments were approved by the ethical committee for animal care (2013/UCL/MD/004). Spinal cord lesions were described (Ansorena et al. 2013; Appendix). Groups of 5 or 6 rats were used (Schira et al. 2012). The animals were given cyclosporine (15 mg/kg, subcutaneous) 1 d prior to the surgery and every day during the entire course of the experiment (Schira et al. 2012) to prevent immunologic responses due to cross-species.

Animals were divided in 5 experimental groups:

- **Group 1:** sham-operated group, where a laminectomy was performed but no spinal cord lesion was made $(n = 5)$.
- **Group 2:** untreated group, where hemisection was performed with no treatment $(n = 5)$.
- **Group 3:** apical papilla group, where the lesion site was filled with 10 μL of fibrin hydrogel, followed by the implantation of a whole apical papilla $(n = 6)$.
- **Group 4:** SCAP-loaded fibrin hydrogel group, where the lesion site was filled with 10 μL of SCAP-loaded fibrin hydrogel $(n = 5)$.
- **Group 5:** fibrin hydrogel group, where the lesion site was filled with 10 μL of fibrin $(n = 5)$.

Fibrin hydrogels were formed by the coinjection of a SCAP-loaded fibrinogen solution $(1 \times 10^7$ SCAP in 5 μL) for group 4 or a fibrinogen solution (5 μL) for group 5 with thrombin (10 μL, final volume; $n = 5$). Rat bladder was emptied twice per day until bladder function recovery (des Rieux et al. 2014). Rat body weight was recorded for each animal over the duration of the experiment.

**Basso-Beattie-Bresnahan Open-field Locomotor Score**

The Basso-Beattie-Bresnahan (BBB) test was performed 1 and 3 d postsurgery (Basso et al. 1995) by 3 independent observers blinded for conditions for a duration of 5 min per rat. The BBB test—to the contrary of the CatWalk, which records the gait of rat and thus provides numbered parameters—is based on condition-blind operators who apply a scoring to each rat based on hind limb movements. The BBB test does not require weight support, as is the case for the CatWalk, and was thus used to evaluate the rat handicap postsurgery. Rats recovered weight support within 1 to 2 wk postsurgery.

**CatWalk Gait Analysis**

Locomotor function was evaluated with the CatWalk gait analysis assay (CatWalk 7; Noldus, Wageningen, Netherlands). Rats were trained daily during 2 wk before the surgery to ensure the quality of the data obtained from the CatWalk analysis. Up to 5 runs were recorded per rat, and analysis was performed on 3 of these runs showing an uninterrupted crossing of the glass runway within 1 to 2 s (Ansorena et al. 2013). Data on gait were acquired and analyzed with the CatWalk 7.1 software program (Deumens et al. 2007). Recovery of weight support is required to be able to perform gait analysis. All animals recovered weight support by the first week after surgery. Gait parameters showing an SCI-induced deficit specific to the ipsilateral side (side of injury) were selected for analysis: stance duration (duration of paw contact with glass runway), swing duration (duration of no-paw contact with glass runway), swing speed (distance covered by step/duration of the step), and paw print area (surface of the paw in contact with the glass runway). All of these parameters were measured as an average for the step
cycles. The ratios between values of ipsilateral (injured) and contralateral (noninjured) sides were calculated to analyze these gait parameters between preoperative time (baseline) and postoperative time (6 wk after surgery).

Mechanical Withdrawal Thresholds (von Frey)

To assess the potential development of mechanical allodynia due to the treatments, the von Frey hair filament test was used before surgery to get a baseline and every week for 6 wk post-injury (Deumens et al. 2008). The up-down method was followed for a total of 5 filament applications to the ipsilateral hind paw after the first aversive response, as described previously (Chaplan et al. 1994). The 0.4- and 15.0-g values were used as lower and upper cutoff values, respectively.

Histology

Six weeks after surgery, spines were collected and treated for histology (Appendix). Sections were processed for Masson trichrome staining. Human cells were identified by immunostaining against the human mitochondrial antigen, and macrophage infiltration and accumulation around the lesion were analyzed by CD68 staining. L4/5 spinal cord tissues were cryosectioned (25 μm) and collected with an intersection distance of 250 μm. Activated microglia were detected by Iba-1 staining. Iba-1 signal was quantified (Fig. 4a) with ImageJ (1.43u; National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

Statistical analyses were performed by GraphPad PRISM (GraphPad Software, San Diego, CA, USA; Appendix).

Results

Impact of Spinal Cord Hemisection on Rat Motor Function and General Health

Sham-operated animals presented a maximal BBB score at days 1 and 3, indicating a normal locomotion. Significant differences were observed between BBB scores of sham-operated animals and injured animals at 1 and 3 d after the surgery. When a hemisection was performed, no coordination (score, <11) was noticed at day 1 or 3, regardless of the group. There was no significant difference between BBB scores of all injured groups (Fig. 1b).

Regardless of the group, the rats followed the same weight curve as naive animals, from ±200 g to ±250 g in 6 wk (Appendix Fig. 1). Animals returned to a normal nutritional habit after the surgery. Urinary function was described as a biomarker of animal health after SCI (Cruz et al. 2015). Immediately after the injury, the detrusor undergoes an areflexic period resulting in urinary retention (spinal shock; Cruz et al. 2015). We observed a spinal shock for all but sham-operated animals. One hundred percent of the injured animals recovered bladder function within 4 d after the surgery (Fig. 1c).

Impact of Treatments on Rat Functional Recovery

All the animals were trained and learned to cross the CatWalk without hesitations and with a run time <2 s. One week after SCI, all animals recovered body weight–supported stepping and were thus operational for CatWalk gait analysis. Stance duration ratios of sham- and apical papilla–treated groups before lesion (BL) and 6 wk after the surgery were equivalent, while stance duration ratios of untreated, hydrogel-treated, and hydrogel-loaded-with-SCAP groups were significantly lower than those before the surgery (Fig. 2a). Both swing duration and swing speed ratios were not different between BL and 6 wk postsurgery only for sham- and apical papilla–treated groups (Fig. 2b, c). Swing duration ratios were 1.7 and 2 times higher at 6 wk postsurgery compared with BL for untreated and hydrogel-treated groups (Fig. 2b), while swing speed ratios were 1.7 and 2 times lower at 6 wk postsurgery than BL (Fig. 2c), respectively. Only apical papilla–treated animals showed print area percentages similar to those of sham-operated animals (Fig. 2d). Thus, rats treated with the apical papilla were the only group that showed no differences between BL and 6 wk for the 4 analyzed parameters. No differences were
sented cystlike cavities and scar tissue at the lesion site (Fig. 5A, (Zhang et al. 2011), all groups with spinal cord hemisection pre-
animals (Fig. 5A, a). As already reported for this kind of lesion
motion of the spinal cord structure was observed for sham-operated
vs. SCAP-treated rats and 2 times less vs. sham, untreated, and
papilla showed significantly reduced microglial activation
groups 6 wk postsurgery (Fig. 4b). Rats treated with apical
Iba-1 staining was observed in the lumbar region (L4-L5) of
inflammatory pain (mechanical threshold of 15.0 g; Fig. 3). Similar
Six weeks postsurgery, no animal demonstrated chronic inflam-
tor function.

Discussion
The first part of the null hypothesis must be rejected, since the
transplantation of a whole apical papilla into a spinal cord hemisection at T10 in a rat model showed significantly
improved motor recovery compared to the control groups. At 6
wk, CatWalk analyses indeed showed that only sham and apical
papilla transplant groups did not show any significant
impairment in stance duration, swing speed and duration, and
print area, as compared with that before the lesion. The second
part of the null hypothesis cannot be rejected, since no signs of
alldynia were revealed in any group.

In this investigation, all the injured animals showed a good
uniformity of the locomotor handicap generated by the spinal
cord hemisection. We also excluded the influence of postoper-
ative malaise on rat functional and sensorial analysis by show-
ing that weight curves were similar to those of naïve animals
and that bladder function recovered quickly after the surgery
(4 d). Besides, the laminectomy had no impact on rat locomo-
tor function.

When implanted into a rat hemisection model, human apical
papilla induced a significant improvement of the rat motor
function. Recovery of hind limb locomotor function injury fol-
lowing injection of dental stem cells after spinal cord section is
in line with a previous report based on SHED (Sakai et al. 2012). However, unlike that work, no positive effect could be
highlighted here when SCAP were delivered in a fibrin hydro-
gel. Such differences can first be explained by different study
designs but also by the choice of the BBB test to evaluate func-
tional recovery over less subjective analysis, such as CatWalk
(Koopmans et al. 2005). A second explanation for the lack of
efficiency of SCAP hydrogel as compared with the apical
papilla transplant is likely related to a fast degradation of the
fibrin hydrogel. After 6 wk, scarcely any remaining human
cells could indeed be detected in the SCAP hydrogel group.
Previous work by our group showed proliferation and low
apoptosis of SCAP incorporated into the presently used fibrin
hydrogel formulation and implanted subcutaneously in the
peritoneum for 1 wk (Germain et al. 2015). However, it appears
insufficient to maintain the SCAP for 6 wk in a spinal cord
hemisection. A third explanation could be the death of the

Impact of Treatments on Chronic
Pain and Response
Six weeks postsurgery, no animal demonstrated chronic inflam-
atory pain (mechanical threshold of 15.0 g; Fig. 3). Similar
Iba-1 staining was observed in the lumbar region (L4-L5) of
rats from sham-operated, untreated, and hydrogel-treated
groups 6 wk postsurgery (Fig. 4b). Rats treated with apical
papilla showed significantly reduced microglial activation
(Iba-1 staining) than that of the other conditions (2.8 times less
vs. SCAP-treated rats and 2 times less vs. sham, untreated, and
hydrogel-treated rats). SCAP-treated rats showed microglial
activation, reflected by a significant increase in Iba-1 staining
as compared with the other conditions (1.5 times vs. sham,
untreated, and hydrogel-treated rats).

Impact of Treatments on Histologic Structure
Masson trichrome staining was used to analyze the spinal cord
tissue morphology at the laminectomy/lesion site. No modific-
ation of the spinal cord structure was observed for sham-operated
animals (Fig. 5A, a). As already reported for this kind of lesion
(Zhang et al. 2011), all groups with spinal cord hemisection pre-
sent cystlike cavities and scar tissue at the lesion site (Fig. 5A,
b–e). For the nontreated group and the hydrogel-treated groups,
human SCAP due to the host response. However, in this study as in others, animals were given cyclosporine, and in similar conditions, the success of cross-species applications has already been demonstrated (Schira et al. 2012; Li et al. 2015).

On the contrary to the hydrogel-treated groups, the apical papilla was still visible in the hemisection site, and its integrity appears to be maintained 6 wk postimplantation, with cells positive for human mitochondria staining. Hence, the positive effect of the apical papilla implantation on functional outcome may be related, not only to the preservation of SCAP in their niche, but also to their maintenance and better protection against the nonfavorable lesion environment provided by the apical papilla tissue. Unlike the dental pulp, most of the cells in the apical papilla present the required mesenchymal stem cell markers (Ruparel et al. 2013), and they possess a high proliferative ability within the tissue (Sonoyama et al. 2008). Their ability to stimulate neurite outgrowth via the secretion of neurotrophic factors (de Almeida et al. 2014) is likely to play an important part in the positive effect observed here. Moreover, it was recently shown that hypoxia upregulates the expression of various growth factors and neurospecific genes (Vanacker et al. 2014), and given the low vascularity of the apical papilla transplant, this is an important factor to consider. Nosrat et al. (2001) demonstrated that the implantation of a human dental pulp fragment into a spinal cord lesion increased the number of rescued motoneurons 4 wk post-injury, which was attributed to glial cell line–derived neurotrophic factor secretion by the pulp cells. However, no histology of the fragment at the lesion site, no specific staining of human living cells, nor functional improvement has been reported. Finally, it must be mentioned that the improved recovery via the apical papilla may be partly due to the physical support provided by the apical papilla that could stabilize the primary injury and slow down the apparition of secondary lesions.

In this study, no chronic pain was observed in the rats, whatever the condition, thereby showing no adverse effect of the hydrogel, SCAP, and apical papilla implantation. Mesenchymal stem cells are known for their immunoregulatory properties (Schubert et al. 2011), and their impact on macrophage/microglia phenotype has been demonstrated (Zanier et al. 2014). Additionally, it has been demonstrated that SHED injected into an injured spinal cord promoted recovery by altering macrophage polarity (Matsubara et al. 2015). Therefore, we investigated the effect of the apical papilla implantation on microglial activation (Ohsawa et al. 2004), and we observed that Iba-1 immunoreactivity was significantly lower for the apical papilla–treated group versus other groups. Although a more detailed analysis would be complimentary, these results suggest that the apical papilla could reduce inflammation and thus provide more favorable conditions leading to an improved functional outcome (Koopmans et al. 2009). These results underline the importance of maintaining the SCAP in their original environment or trying to mimic it as well as possible for transplantation strategies.

In conclusion, transplantation of a human apical papilla at the lesion site improves gait in rats 6 wk after SCI without inducing chronic pain and reducing glial reactivity, while SCAP-hydrogel-treated rats showed no improvement. We did
not expect the lack of efficiency of isolated SCAP considering the results obtained in previous studies (Sakai et al. 2012). There are several possible reasons why we did not observe an impact on the gait of rats, among which is the inadequacy of the hydrogel, although previous in vitro results (Germain et al. 2015) were encouraging but on a shorter timescale due to in vitro limitations. Rather than serving as a direct comparison, the distinct outcomes between the 2 intervention strategies serve as an important message: dental sources may have a place in an interventional approach to improve SCI, but beneficial effects may depend on the specific way by which the tissue/cells are delivered. These results also underline the potential of delivering SCAP in their original niche. To the extent of our knowledge, this is the first work describing a human apical papilla implantation into an SCI, but further work is now required to explore the underlying mechanisms of the apical papilla efficiency, notably regarding secretory activity of SCAP in the transplanted papilla but also to improve SCAP delivery.

**Author Contributions**

P. De Berdt, contributed to conception, design, data acquisition, analysis, and interpretation, drafted the manuscript; J. Vanacker, contributed to conception, design, data acquisition, and analysis, critically revised the manuscript; B. Ucakar, contributed to data acquisition and analysis, critically revised the manuscript; L. Elens, contributed to data analysis, critically revised the manuscript; A. Diogenes, contributed to data interpretation, critically revised the manuscript; J.G. Leprince, contributed to conception, design, and data interpretation, drafted and critically revised the manuscript; R. Deumens, A. des Rieux, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

**Acknowledgments**

The authors acknowledge the dental clinic Vastega BVBA (Kraainem, Belgium) and their patients for providing the wisdom teeth. The Tisseel fibrin sealant kit was kindly provided by Baxter Innovations GmbH (Vienna, Austria). The authors thank Dr. Olivier Schakman (Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium) for access to and help with the CatWalk. Oliver Schakman is a recipient from the Walloon Region’s Marshall Program of Excellence (DIANE Convention). We are also grateful to Fonds National de la

Figure 5. Spinal cord lesion histologic structure. (A) Masson trichrome staining was used to analyze the spinal cord tissue morphology at the laminectomy/lesion site. (a) Sham, (b) untreated, (c) apical papilla, (d) SCAP-loaded fibrin hydrogel, and (e) fibrin hydrogel. (B) Human cells were identified by immunostaining against the human mitochondrial antigen (brown). (a, b) Apical papilla and (c, d) SCAP-loaded fibrin hydrogel. Image acquisition was performed with a Leica SCN400 slide scanner. SC, spinal cord; SCAP, stem cells of the apical papilla. This figure is available in color online at http://jdr.sagepub.com.
Recherche Scientifique and Université Catholique de Louvain (Fonds de la Recherche Scientifique) for the financial support. A. des Rieux is a research associate; R. Deumens, a scientific collaborator; and J. Vanacker is a postdoctoral researcher at the Fonds de la Recherche Scientifique / Fonds National de la Recherche Scientifique. J.G. Leprince thanks the Fondation St. Luc for his research fellowship. The authors are recipients of subsidies from the Fonds National de la Recherche Scientifique / Fonds National de la Recherche Scientifique Médecale. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References


