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Surgical access to the mammalian cochlea for cell-based therapies

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Abstract

Cochlear implants are dependent on functionally viable spiral ganglion neurons (SGNs) – the primary auditory neurons of the inner ear. Cell-based therapies are being used experimentally in an attempt to rescue SGNs from deafness-induced degeneration or to generate new neurons. The success of these therapies will be dependent on the development of surgical techniques designed to ensure precise cell placement while minimizing surgical trauma, adverse tissue reaction and cell dispersal. Using 24 normal adult guinea pigs we assessed three surgical procedures for cell delivery into the cochlea: (i) a cochleostomy into the scala tympani (ST); (ii) direct access to Rosenthal's canal – the site of the SGN soma - via a localized fracture of the osseous spiral lamina (RC); and (iii) direct access to the auditory nerve via a translabyrinthine surgical approach (TL). Half the cohort had surgery alone while the other half had surgery combined with the delivery of biocompatible microspheres designed to model implanted cells. Following a four week survival period the inflammatory response and SGN survival were measured for each cohort and the location of microspheres were determined. We observed a wide variability across the three surgical approaches examined, including the extent of the inflammatory tissue response (TL>>RC>ST) and the survival of SGNs (ST>RC>>TL). Importantly, microspheres were effectively retained at the implant site after all three surgical approaches. Direct access to Rosenthal's canal offered the most promising surgical approach to the SGNs, although the technique must be further refined to reduce the localized trauma associated with the procedure.

Keywords

Deafness; stem cell; cell-based therapy; auditory nerve; cochlear implant; inner ear

INTRODUCTION

The World Health Organization estimates that 278 million people suffer from a moderate to profound hearing loss in both ears (40). The great majority of these patients will have a sensorineural hearing loss (SNHL) typically affecting the sensory hair cells of the cochlea. While avian hair cells are capable of regeneration after trauma (8,29), hair cells within the mammalian cochlea cannot regenerate. Moreover, their death causes a subsequent progressive

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degeneration of the primary afferent auditory neurons, the spiral ganglion neurons (SGNs) (20,35).

The only therapeutic intervention for patients with a severe to profound SNHL is a cochlear implant – a neural prosthesis designed to electrically stimulate residual SGNs (2). Since cochlear implants are dependent upon functionally viable SGNs, a number of experimental procedures are being developed in an attempt to maintain or increase the number of SGNs within the deafened cochlea. Short-term maintenance of surviving SGN populations has been achieved in animal models of SNHL by local application of exogenous neurotrophins (9,10, 24,25,28,33,36,39,43), or via gene transfer therapy using viral vectors (19,42). In addition, several studies have now investigated the potential of stem cells to replace the degenerating population of SGNs following SNHL in various animal models (6,7,15,38). Although the delivery site and method for transplantation of cells is likely to vary depending on whether the cell therapy is designed to deliver neurotrophins or is for cell replacement of SGNs, the underlying surgical procedure must not compromise residual SGNs or evoke an adverse inflammatory response within the cochlea.

Several techniques have been used to deliver live cells into various locations within the mammalian cochlea (Fig. 1). These techniques include (but are not limited to) the scala media (13), the scala tympani (6,16,17), the auditory nerve or modiolus (7,15,27,31), Rosenthal's canal (4) and the semicircular canals of the vestibular system (18). Each technique confers specific advantages to the overall aim of each study, but each technique also has its own disadvantages. While less invasive techniques (for example scala tympani or semi-circular canal approaches) may limit damage to the existing cochlear structures, they tend to result in cell dispersal (6). Conversely, more invasive techniques (such as auditory nerve or modiolus approaches) may limit cell dispersal but cause increased levels of damage. This additional damage may include the initiation of an inflammatory tissue response, and further degeneration of the residual SGNs.

An important aspect in the progress of cell-based therapies for SNHL is the development of clinically relevant surgical techniques which are designed to incorporate precise cell placement while minimising surgical trauma and inflammation in the cochlea. Here, we assess the safety and efficacy of three potential surgical procedures for the cell delivery into the cochlea, by comparing the effects of surgery alone and surgery in combination with biocompatible microspheres to model exogenous cells. The three surgical routes evaluated are illustrated in Figure 1 and include (I) the scala tympani via a basal turn cochleostomy (ST); (II) direct access to Rosenthal's canal via a localised fracture of the osseous spiral lamina wall in the lower basal turn scala tympani (RC); and (III) direct access to the auditory nerve via a translabyrinthine approach (TL; 14). Each approach was evaluated in terms of the retention of microspheres at the surgical site, the degree of inflammation detected within the cochlea, and the density of endogenous SGNs one month after treatment.

METHODS

All animal handling and experimental protocols were approved by the Animal Research & Ethics Committee, Royal Victorian Eye and Ear Hospital, Melbourne (approval number 05/122A) and complied with the NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Study overview

Twenty four normal hearing adult guinea pigs weighing 575 ± 53 g (mean $\pm$ SD) with an equal sex distribution and taken from a mixed gene pool were used in this study. The animals

were randomly assigned to one of 6 surgical experimental groups (n=4 for each group; Table 1). Surgery was performed unilaterally; the right cochleae formed un-operated controls.

For each surgical approach, one cohort received microspheres in a hydrogel matrix (Groups 1, 3 & 5; Table 1) while the second cohort received surgery alone (i.e. without delivery of microspheres and hydrogel; Groups 2, 4 & 6; Table 1).

Otoscopic examination and hearing status

All guinea pigs exhibited otoscopically normal external and middle ears and hearing status was confirmed normal by recording auditory brainstem responses to 43 dB SPL click stimuli (12). These procedures were performed while the animals were under general anaesthesia (Ketamine, 40 mg/kg; Xylazine, 4 mg/kg, co-administered intramuscularly [i.m]).

Preparation of microspheres in hydrogel

Cibacron blue microspheres (Sigma) were used to model exogenous cells. These spherical microspheres can be readily identified using light microscopy. The microspheres range in size from 40 – 140 μ m, are biocompatible and designed specifically to be used as tracer beads for *in vivo* experiments.

Twenty four hours prior to surgery, the microspheres were encapsulated in liquid hydrogel (BD Biosciences) under sterile conditions. The hydrogel was used as a biocompatible matrix to minimize the dispersal of the microspheres following implantation. A 5 μ L volume of hydrogel was placed into a sterile Petri dish, mixed with 5 μ L of microspheres and allowed to stand for 5 minutes. Using a sterile transfer pipette, 1–2 mL of phosphate buffered saline (PBS, pH 7.4) was used to harden the microsphere/hydrogel mixture, according to the manufacturer's specifications. After five minutes at room temperature the PBS was removed, a Petri dish containing the microsphere/hydrogel mixture was sealed and stored at 4°C overnight. Several preparations of microspheres encapsulated in hydrogel were prepared for each guinea pig in Groups 1, 3 and 5 (Table 1).

Surgery

Surgical levels of anesthesia were induced using Ketamine (60 mg/kg, i.m.) and Xylazine (4 mg/kg, i.m.). Respiratory tract secretions were reduced using Atropine sulphate (0.06 mg, i.m.). Local anaesthetic (0.2 ml Lignocaine hydrochloride 2%, administered subcutaneously [s.c.]) was injected along the surgical incision site. Pre-operative analgesia using Carprofen (4 mg/kg, s.c.; diluted 1:10 with 0.9% sodium chloride) was followed by post-operative antibiotic Enrofloxacin (10 mg/kg, s.c.) and fluid replacement with Hartmann's solution (10 ml, s.c.).

An experienced Otologist performed all surgical procedures using sterile surgical techniques. Each guinea pig was placed on an operating table maintained at 37°C. The left auditory bulla was exposed then opened to provide a clear view onto the lateral wall of the basal turn scala tympani and the bony prominence of the posterior semicircular canal (41). Further surgery was performed using an operating microscope and a high speed 0.6 mm diameter diamond burr drill with saline irrigation.

Soft surgery techniques (22) were used to drill a cochleostomy into the basal turn scala tympani, ensuring maintenance of an intact 1 mm bony rim around the untouched round window membrane (Fig. 2). To access Rosenthal's canal, a cochleostomy was performed to visualise the basal turn scala tympani and the thin osseous spiral lamina covering Rosenthal's canal was fractured using a tungsten microelectrode with a tip diameter < 5 μ m (TM33B01; World Precision Instruments). For the translabyrinthine approach (TL), the bony ridge of the posterior semicircular canal inferior and posterior to the base of the cochlea was identified (1) and the

canal lumen was drilled medially to the vestibule. Drilling the medial wall of the vestibule caudal to the cochlea (41) exposed the auditory nerve within the internal auditory meatus.

Once the respective cochlear locations were exposed a single droplet of the microsphere/hydrogel mixture was delivered to the surgical site (Groups 1, 3 and 5; Table 1) via the fine end of a 16 cm straight micro-needle (Kaisers KAEN2171) used routinely to deliver the microsphere/hydrogel mix for all three treatment groups. This procedure was repeated and the second droplet delivered to a glass slide. The number of beads were counted under a microscope to provide an estimate of the number of microspheres delivered to each cochlea. Animals in cohorts 2, 4 and 6 (Table 1) received identical surgery *without* delivery of microspheres or hydrogel. The cochleostomy or posterior semicircular canal bony defect was sealed with a muscle plug and the skin wound was closed with sutures (Fig. 2).

Histological techniques

The guinea pigs were euthanized four weeks following surgery via an intraperitoneal injection of sodium pentobarbitone (160 mg/kg) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, (pH 7.2–7.4). Both cochleae were removed and a small hole was created at the apex of the cochlea to allow rapid penetration of fixative over a two hour period. The cochleae were decalcified in 10% ethylenediamine tetra-acetic acid in 0.1M PBS (pH 7.2–7.4) until decalcified and then trimmed, embedded using Tissue Tek OCT compound (ProSciTech) and cryosectioned at 12 μm (3). Serial sections were mounted onto super-frost slides (Fisherbrand), air-dried for 2 hours and then transferred to -80°C for storage. All sections were stained with Hematoxylin & Eosin. To minimize the potential for bias we selected twenty sections randomly throughout each treated cochleae for quantitative analysis of both inflammatory tissue response and SGN density. Ten sections were also randomly selected for analysis from each untreated control cochleae.

Inflammatory tissue response

The nature and extent of any inflammatory tissue response was examined in the present study. Both areolar fibrous tissue and new bone were identified histologically and the extent of the response was determined quantitatively by measuring the area of any inflammatory tissue response within the scala tympani, scala media and scala vestibuli of the lower basal turn (LB), upper basal turn (UB), turn 2 (T2), turn 3 (T3) and turn 4 (T4) using NIH image. The area of inflammatory tissue response (in μm^2) for each of these five cochlear locations was summed for each cohort. No attempt was made to differentiate the nature of the inflammatory tissue response, i.e. areolar fibrous tissue and new bone were treated identically.

Spiral ganglion neuron density

Neural survival was quantified by calculating SGN density (cells/ mm^2) in Rosenthal's canal of the LB and UB turns of both the treated (left) and control (right) cochleae of each cohort. The number of SGNs with a visible nucleus was divided by the cross-sectional area of Rosenthal's canal measured using NIH image. In addition, SGN density was measured in histological sections containing the surgical site of access to Rosenthal's canal (RC-SS; Groups 3 and 4; Table 1) in order to determine the localized neural loss associated with this surgical procedure. Spiral ganglion neuron densities for each surgical approach (ST, RC, TL) were compared statistically with the corresponding regions from their contralateral control cochleae using the non-parametric Kruskal-Wallis One-way analysis of variance.

Microspheres: number implanted and dispersal

The microspheres were counted at seven anatomical locations within each of the 20 sections per cochlea examined in Groups 1, 3 and 5: LB; UB; T2; T3; T4; auditory nerve (AN) and the

labyrinth (L). The total number of beads within each of these anatomical sites was determined for each experimental group.

RESULTS

Distribution of microspheres

Analysis of the samples of microspheres collected during surgery indicated that there was no significant difference between the numbers of microspheres implanted in each cohort (mean 210 ± 31 SEM). Microspheres were readily identified by light microscopy (e.g. Figs. 3 & 6) and were all contained within the implanted left cochleae (Groups 1 & 3) or the auditory nerve and labyrinth (Group 5). No microspheres were detected in the contralateral control cochleae.

All microspheres within the ST group were located within the scala tympani (e.g. Fig. 3). The great majority of microspheres were retained in the LB ($n=275$) and UB ($n=87$) turns, although small numbers were located in T2 ($n=2$) and T4 ($n=3$) in one cochlea (Fig. 4).

All microspheres within the RC group were also located within the scala tympani (e.g. Fig. 3). Due to the relatively restricted surgical opening into Rosenthal's canal ($\sim 80 \mu\text{m}$), the microspheres were typically observed adjacent to, but not within, Rosenthal's canal. The vast majority of microspheres were retained in the LB turn ($n=379$), although isolated microspheres were also present in the UB turn ($n=1$), T2 ($n=2$) and T3 ($n=1$; Figs. 4 & 6).

The majority of microspheres within the TL group were retained at the implant site, either within the labyrinth ($n=290$) or on the auditory nerve ($n=30$; Fig. 6). However in one cochlea microspheres were observed in the scala media of the LB turn ($n=15$; Fig. 4).

Inflammatory tissue response

The nature and extent of the inflammatory response observed in the present study varied both spatially within each cochlea and across surgical techniques. Representative photomicrographs illustrating the typical tissue response to each of the three surgical techniques are shown in Fig. 3. In each example the surgery included the delivery of microspheres. The extent of inflammation evoked by either surgery alone or surgery with implantation of microspheres in hydrogel for each of the three different surgical approaches is illustrated in Fig. 5. There was no inflammatory tissue response evident in any of the right (untreated) contralateral cochleae.

Surgical access to the scala tympani (ST; Groups 1 and 2) typically elicited a small inflammatory response in the treated cochleae consisting of localized new bone formation and fibrous tissue. In all cases this inflammatory response was limited to the LB turn in the vicinity of the cochleostomy (Fig. 5). For the surgery alone cohort (Group 2), three of the four cochleae exhibited a mild inflammatory tissue response limited to a partial filling of the scala tympani within the LB turn. A similar localized inflammatory response was evident in all four cochleae that had undergone surgery with microsphere implantation (Group 1). There was no evidence that the inflammatory response had extended beyond the LB turn or into the scala media or scala vestibuli. Moreover, the similarity in the extent of the inflammatory response for both cohorts (Fig. 5) suggests that the presence of microspheres and hydrogel did not contribute appreciably to the tissue response.

Surgery to expose Rosenthal's canal (RC; Groups 3 and 4) elicited a localized mild to moderate inflammatory response in all eight treated cochleae (Fig. 5). This response also consisted of both new bone and fibrous tissue, and was centred at the surgical sites associated with the cochleostomy and the localized fracture of the osseous spiral lamina. In all but one cochlea the inflammatory response was localized to the LB turn; in the remaining case a slight tissue response had extended into the UB turn. There was no evidence of an inflammatory response

extending into more apical turns of the cochleae or into the scala media or scala vestibuli (Fig. 5). In this surgical approach the inflammatory response was more extensive in the microsphere/hydrogel cochleae than the surgery-only cochleae.

The translabyrinthine approach to expose the auditory nerve (TL; Groups 5 and 6) elicited a wide range of inflammatory responses. These responses included three cochleae exhibiting a widespread fibrous tissue reaction occupying all three scalae from the cochlear base to T3 (e.g. Fig 3C), while a similar number of cochleae showed no evidence of an inflammatory response (e.g. Fig 6A). The remaining cochleae in the TL cohorts showed a localized tissue response and were predominantly located within the scala media of the UB turn although an inflammatory response was also present in the scala vestibuli and scala tympani (Fig. 5). Only cochleae associated with translabyrinthine surgery exhibited an inflammatory reaction within the scala media and scala vestibuli. Moreover, these cochleae only exhibited a fibrous tissue response; new bone formation was not observed. Finally, the presence of microspheres and hydrogel did not contribute to the extent or distribution of this tissue response (Fig. 5).

Spiral ganglion neuron density

The extent of SGN survival in the LB and UB turns of the treated cochleae compared with their respective contralateral controls is illustrated in Fig. 7. Within the LB turn, all cochleae associated with RC surgery (Groups 3 & 4) exhibited a highly statistically significant reduction in SGN density. This reduction was localized to the Rosenthal's canal surgical site (RC-SS; Fig. 7) when compared with SGN density at adjacent sites within their respective contralateral control cochleae. The loss of SGNs was typically localized to the region of Rosenthal's canal that had been opened during surgery. With one exception (LB turn RC Surgery; Fig. 7B), SGN populations distal to the surgical site were not significantly reduced. There was no statistically significant reduction in SGN density in the UB region of cochleae in the RC cohorts. The TL cohort exhibited a large variability in SGN loss. In both cohorts (Groups 5 and 6) the reduction in SGN density was highly statistically significant compared with untreated contralateral control cochleae. Importantly, similar levels of SGN loss were evident irrespective of microsphere/hydrogel implantation (Fig. 7), indicating that the loss of SGNs was most likely associated with trauma as a result of the surgery. Unlike the ST and RC surgical approaches, the TL approach produced significant SGN loss in both the LB and UB turns. There was no statistical difference in SGN density for cochleae subject to ST surgery (Groups 1 and 2) compared with untreated control cochleae for both LB and UB turns (Fig. 7).

DISCUSSION

We evaluated the safety and efficacy of three potential surgical techniques for cell delivery into the inner ear by comparing the effects of surgery alone and surgery in combination with biocompatible microspheres to model exogenous cells. The study demonstrated that hydrogel was effective at retaining microspheres at their implant site, suggesting that this biocompatible, three dimensional matrix is capable of preventing the widespread dispersal of exogenous cells delivered to the cochlea which has been reported previously (6). Widespread cell dispersal can present a number of disadvantages including reducing the number of cells reaching the therapeutic site of interest (RC and the SGNs in this example); potentially evoking a widespread tissue reaction within the cochlea and spreading to the central nervous system via a patent cochlear aqueduct (11). On the other hand, once positioned at their target site, it is important for stem cells to migrate and form functional connections. Although hydrogel provides a temporary scaffold *in vivo*, the effects of this matrix on neurite growth is unknown. Finally, although not explored in the present study, hydrogels could incorporate neurotrophins or other factors designed to promote cell survival, assist cell differentiation, and/or promote neurite growth for cells within the matrix (e.g. 26).

The ST surgical approach evoked a minimal fibrous tissue response and new bone was restricted to a small region of the scala tympani adjacent to the cochleostomy. This technique showed no evidence of loss of SGNs and there was minimal dispersal of implanted microspheres. The surgery is not technically difficult and the procedure already has extensive clinical use associated with cochlear implantation. The drawback of the technique is that it does not specifically target the SGNs within Rosenthal's canal – few cells delivered into the scala tympani are likely to migrate into Rosenthal's canal (6), although it should be noted that the osseous spiral lamina has an extensive network of fine pores through which drugs and cells can potentially move (23,30,34). The ST delivery method is therefore likely to be most useful to groups interested in the local application of drugs into the cochlea.

The RC approach evoked a moderate fibrous tissue response although this was localized to the surgical site in the LB turn of the cochlea. While slightly more extensive than observed following the ST surgical approach, new bone formation was localized to the scala tympani associated with both the cochleostomy and the surgical entry point into Rosenthal's canal. This surgical exposure of Rosenthal's canal also produced a decrease in SGN density, however like the tissue response, this neural loss was restricted to the surgical site – a finding also observed following trauma to this structure during cochlear implantation (21,32,37). We observed minimal dispersal of microspheres from the surgical site with this procedure. Refinements to both the surgical and delivery techniques described are likely to result in further improvements to this approach by way of reducing the trauma to the osseous spiral lamina and adjacent SGNs. To this end, Sekiya and colleagues (31) have described the use of fine glass pipettes (50–100 µm tip diameter) to effectively deliver cells into Rosenthal's canal with minimal trauma. This approach therefore offers an opportunity to deliver cells directly into Rosenthal's canal, the target site for stem cell-based neuronal replacement therapy (5).

The TL approach to the auditory nerve evoked a variable but often widespread fibrous tissue response throughout all turns of the cochlea. There was no evidence of new bone formation with this surgical approach, although given longer survival periods it is possible that the fibrous tissue response observed in these cochleae would resolve into new bone. An extensive tissue response was associated with a significant reduction in SGN density throughout the cochlea. Like ST and RC surgery, TL surgery resulted in minimal dispersal of microspheres. Although this approach is frequently used to approach the auditory nerve in humans, it is technically difficult and usually sacrifices any residual preoperative cochlear function. In our opinion this technique would require significant refinement before it could be considered a suitable surgical approach for cell transplantation therapies.

It is important that the surgical technique developed for therapeutic cell delivery to the inner ear evokes minimal fibrosis or osseoneogenesis as these inflammatory responses could be expected to effect cell migration and neurite outgrowth. Moreover, in procedures involving release of neurotrophins, a vigorous tissue growth may adversely affect the distribution of these drugs within the inner ear. Finally, it is not surprising that we observed new bone formation in cochleae with direct surgical intervention as trauma to the osseous spiral lamina and the endosteal lining of the inner ear has been shown to evoke osseoneogenesis (21,32).

When developing new methods for the transplantation of exogenous cells into the mammalian cochlea, it is imperative to consider the clinical relevance of these delivery strategies and include quantitative analyses of inflammation and density of endogenous neurons. In all cases, surgery to rescue or replace degenerating SGNs must not cause more damage than originally present.

Conclusion

The present study assessed the safety and efficacy of three potential surgical procedures for cell delivery into the inner ear with the objective of targeting the SGNs within RC. We observed a wide variability across the three surgical approaches examined for both inflammatory tissue response (TL>>RC>ST); SGN density (ST>RC>>TL) and degree of surgical difficulty (TL>>RC>ST). Importantly, microspheres were effectively retained at the implant site in all three surgical approaches presumably as a result of the use of a hydrogel matrix. Moreover, no contralateral control cochleae from the six experimental groups exhibited an inflammatory tissue response or contained microspheres, providing further evidence for the minimal dispersal associated with this technique. Direct surgical access to Rosenthal's canal offers the most promising surgical approach to SGNs, although the technique must be refined to reduce the extent of trauma and the resultant tissue response observed in the present study. On the basis of this study we do not recommend a TL surgical approach, but note that on some occasions access to the auditory nerve can be achieved using this surgical route without undue damage to the cochlea.

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Figure 1.

Schematic diagram illustrating the basic anatomy of the mammalian cochlea in transverse section. The fluid filled cochlea is embedded in the temporal bone of the skull (tb). Each cochlear turn contains three distinct anatomical compartments. The scala tympani (st) and scala vestibuli (sv) contain perilymph, an ultra-filtrate of cerebrospinal fluid; while the scala media (sm) contains endolymph. The auditory nerve (an) is located in the central core or modiolus of the cochlea, while their cell bodies – the spiral ganglion neurons – are located in Rosenthal's canal (rc) a small opening within the bony modiolus that spirals from the cochlea base to the apex. The three surgical approaches used in this study are illustrated; (i) ST – delivery into the lower basal turn scala tympani via a cochleostomy; (ii) RC – delivery directly into Rosenthal's canal in the lower basal turn via a cochleostomey and a localised fracture to the osseous spiral lamina – the thin bony lamina separating Rosenthal's canal from the scala tympani (arrowhead); (iii) TL – translabyrinthine approach directly to the auditory nerve.

Figure 2.

Surgical approach to the guinea pig cochlea for Groups 1–4 of the present study. (A) The cochlea was exposed via a postauricular approach. Double arrowhead illustrates the round window of the cochlea. (B) A cochleostomy was made in the lateral wall of the lower basal turn at the level of the scala tympani (arrowheads). (C) Blue microspheres (arrow) were delivered into the scala tympani in a hydrogel matrix. (D) Blue microspheres (arrow) located in the scala tympani before the cochleostomy was sealed. Scale bar = 600 μm .

Figure 3. Representative photomicrographs illustrating the typical tissue response following surgery and transplantation of microspheres following surgical access to (A) ST; (B) RC; and (C) TL. Microspheres are illustrated in the scala tympani (A & B; arrowheads). Tissues are stained with haematoxylin and eosin (H & E). Scale bar = 500 μ m.

Figure 4. Number of microspheres in the left (implanted) cochleae in cohorts 1 (ST); 3 (RC) and 5 (TL). No microspheres were observed in the contralateral control cochleae. Lower basal turn (LB); Upper basal turn (UB); turn two (T2), turn three (T3); turn four (T4); auditory nerve (AN); labyrinth (L).

Figure 5.

Comparison of inflammatory tissue response (in mm²) in the three surgical groups: scala tympani (ST), Rosenthal's canal (RC) and auditory nerve (translabrynthine; TL), both with microspheres (surgery and hydrogel; left) and surgery alone (surgery; right). Inflammation was restricted to the surgical site using the ST and RC approaches. The TL approach evoked a quite variable inflammatory response that often resulted in a widespread tissue reaction occupying several turns of the treated cochleae. Legend: LB = lower basal turn; UB = upper basal turn; T2, T3, T4 = turn two, turn three and turn four, respectively.

Figure 6.

Photomicrographs illustrating the range in tissue response observed in cochleae that have undergone TL surgery. (A) A cochlea exhibiting a minimum tissue response following transplantation of microspheres via a TL approach (Group 5). A number of microspheres can be seen close to the auditory nerve (AN: arrowhead). (B) A cochlea without microspheres (Group 6) illustrating an extensive tissue response throughout T1, T2 and T3 following TL surgery. This tissue reaction was most extensive in the scala vestibule (sv) and scala media (sm) of T1 and T2 while the scala tympani (st) of T1 was free of a tissue response (*). This cochlea also exhibited a widespread loss of SGNs.

Figure 7.

Comparison of SGN density in the surgical groups: scala tympani (ST), Rosenthal's canal (RC), Rosenthal's canal surgical site (RC-SS), auditory nerve (translabrynthine; TL) for both (A) surgery with microspheres and hydrogel, and (B) surgery alone. SGN density was measured in the lower basal (LB) and upper basal (UB) turns of all cochleae and compared statistically with similar measures from their contralateral control cochleae (not illustrated). Median, inter-quartile range and the 5 and 95 percentile are illustrated. * = $p \leq 0.05$.

Table 1

Experimental design.

	Surgical Approach	Microspheres in hydrogel
Group 1 (n=4)	Scala Tympani	Implanted
Group 2 (n=4)		No implant
Group 3 (n=4)	Rosenthal's Canal	Implanted
Group 4 (n=4)		No implant
Group 5 (n=4)	Translabrynthine	Implanted
Group 6 (n=4)		No implant

Note: All animals had normal hearing prior to surgery. All animals were euthanized four weeks following surgery.