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Periodontal ligament on pulp-free root slices – an in vitro model for early tooth (re)integration

An exploratory study

KEYWORDS
Reintegration of teeth
Ex vivo root slices
Periodontal ligament (PDL)
PDL wound healing
Holistic in vitro model

SUMMARY

Reintegration of freshly extracted healthy teeth is very successful, most likely due to the regenerative capacity of their roots' residual periodontal ligament (PDL). We hypothesised that in vitro cultures of the consecutive slices of a sectioned root will represent the entity of PDL cell types engaged in tooth-sided reintegration.

For confirmation, apex and pulp from human premolars were removed and roots cut into 6 to 9 about 1mm thick slices. These were immobilised to separate wells and cultured for 20 days, under daily inspection for the initiation of cell outgrowth (ICO). ICO and the distribution of vital slices along, the cell growth around as well as the expansion of outgrown cells off the root axes after 20 days were displayed for each tooth as 3D-like profiles. Of the 81 slices from 11 teeth, 55 showed ICO; 64% within one week and 96% within two weeks. Such dynamics compare to the early (day 2–5) and the intermediate (day 9–14) integration phase reported for PDL cells in vivo. Experimental phase contrast images of a single slice showed at ICO few fibroblast- and stem/progenitor-like cells. Four and five days later at the same site cells had grown in number and changed in shape and space over time.

This exploratory study indicates that in root slice cultures PDL cells behave similarly to those during reintegration in vivo. It favours our hypothesis, which is now to be adequately verified. Eventually, the model may facilitate the identification of outgrowing cells and cellular changes over time, as triggered by tissue rupture. It may further allow for emulating cellular interactions between the root surface and alveolar bone or engineered constructs, natural or engineered scaffolds, or other tissue, in an in vivo-like situation.

Introduction

The periodontal ligament (PDL) interlinks a tooth's root surface with the alveolar bone (Beertsen et al. 1997; Nanci & Bosshardt 2006). Tooth extraction ruptures the PDL and its vasculature and stimulates in the residual PDL a.o. stem/progenitor cells to initiate reattachment and PDL restoration (Gould et al. 1977; Pitaru et al. 1994, 2014; Sato et al. 2010). Numerous serial case studies document that freshly extracted and reinserted healthy autologous teeth have a high chance to regain function (Andreasen 1990; Cross et al. 2013; Yoshino et al. 2013). Prerequisites are that i) reinsertions are done at once or upon appropriate storage (Andreasen & Kristersson 1981; Temmerman 2006; Malhotra 2011), and that ii) mature roots are endodontically treated (Schwartz & Andreasen 1988).

Also, teeth affected by periodontitis (PED) have been successfully replanted or substituted (Rouhani et al. 2011). This was either done by intentionally rotating teeth upon extraoral PED-treatment and re-insert them with their treated site to face healthy alveolar PDL (Kawamani et al. 2001), or by replacing PED-affected teeth with healthy autotransplants, which had their PDL tissue augmented by an extraction/reinsertion cycle at the donor site, prior to transplantation (Gault et al. 2002).

The key to a tooth's successful reinsertion lies obviously in the presence of a freshly ruptured, healthy residual PDL lining an intact root surface (Melcher 1976; Andreasen et al. 1981; Tsukiboshi 2002). There, periodontal fibroblast-like (PDF) cells are most abundant (Lekic & McCulloch 1996) and commonly in presence of minor amounts of a.o. cementoblasts, osteoblasts and stem/progenitor cells (Piche et al. 1989; Pitaru et al. 1994, 2014; Lekic & McCulloch 1996; Sato et al. 2010). Cells with mesenchymal stem cell- and pericyte-like properties have been located in perivascular niches (Gould et al. 1977; Iwasaki et al. 2013). They can differentiate into cemento- and osteoblasts, and periodontal progenitor- and PDF-type cells, and replenish the residual PDL (Gould et al. 1977; Roguljic et al. 201v3; Pitaru et al. 2014).

In summary, cell types in the residual PDL have been found heterogeneous. Due to their plasticity, some of these types are apt to re-establish at least any of the mesenchymal lineages involved in the reintegration of extracted teeth (Melcher 1976, 1985; Aukhil et al. 1990; Lekic & McCulloch 1996; Seo et al. 2004; Sato et al. 2010; Pitaru et al. 2014).

Re- or transplantations of autologous teeth are restricted to situations, where suitable teeth are available, i.e. they are not general solutions (Park et al 2010). With more than 10% of the global population suffering from severe PED with teeth prone to loss (Richards 2014), it is not surprising, that for dental research periodontal healing and restoration of compromised teeth is still a major challenge (Aukhil et al. 1990; Susin & Wikesjøe 2013; Haemmerle & Giannobile 2014; Cochran et al. 2015; Lin et al. 2015). The same holds true for appropriate in vitro (Weinreb & Nemcovsky 2015) and in vivo (Bright et al. 2015; Yan et al. 2015) models mimicking regeneration, restoration or reconstruction of a compromised periodontium complex. Notwithstanding substantial progress in research, the achieved results seem not yet to meet all expectations (Aukhil et al. 1990; Susin & Wikesjøe 2013; Lin et al. 2015).

At this point, it might be appropriate to refocus on the long-standing and successful autotransplantation of healthy teeth (Cross et al. 2013). There, functional repair or regeneration initiates with tooth extraction (Melcher 1985; Pitaru et al. 1994, 2014; Lekic & McCulloch 1996; Susin & Wikesjøe 2013; Lin et al. 2015). The rupture of the PDL and its vasculature triggers a cascade of cellular and molecular events, similar to those engaged in mesenchymal tissue repair, as initiated by simple, acute, mechanical injury (Weiss 1961; Sato et al. 2010; Greaves et al. 2013; Dickinson et al. 2013).

In this context, it should be noted that the origin and the differentiation pathways of the cells involved in restoring the ruptured PDL are different from tooth anchoring during odontogenesis (Gould et al. 1977; Melcher 1985; Pitaru et al. 1994, 2014; Susin & Wikesjøe 2013). Accordingly, to follow in vitro the fate of PDL cells on the root surface of freshly extracted teeth over time could be a base, when investigating tooth integration, or impaired periodontal healing, respectively. Such cellular events will not only proceed in the residual PDL lining the root surface of intact re- or transplants. Conceivably, they may also manifest in the PDL lining the mantle surface of the root slices, consecutively cut from a pulp-ectomised tooth, where interference by pulpa cells should be minimal.

Boll (Thesis 1994) and Pohl et al. (1999) had used the activity of PDL cells on pulp-free root slices as a measure of the quality of storage solutions. Accordingly, such root slice cultures were also used by Weiss (2013) for his thesis. He improved the model to allow for non-invasive monitoring over time, and used it to assess the impact of cryopreservation and the length of cryostorage on the vitality of prospective autologous replants. Slices were inspected daily for first outgrowing cells until day 20 in culture, when he registered expansion of outgrown cells by a multiple score system. In confirmation of earlier observations on cryostored teeth and cells (Temmerman 2006). Weiss (2013) noted in slices from cryopreserved teeth a delay in cell outgrowth with respect to that in the freshly extracted controls, though the expansion of outgrown cells was comparable.

The clinical relevance of the root-slice model was explored by reassessing his raw data of the slices from the freshly extracted control group. I condensed the data to objective measurements and binary data sets documenting for each slice i) the days in culture until first cells, and ii) the expansion of out-grown cells at day 20. The first outgrowth dynamics of PDL cell in slice cultures compared to that retrieved by histology from the root surface of reinserted teeth (Temmerman 2006; Sato et al. 2010; Panzarini et al. 2013; Dickinson et al. 2013). Eventually, first cell outgrowth and cell expansion at day 20 were displayed for the individual teeth and its slices as 3D-like profiles.

Materials and Methods

Human premolars from donors undergoing extraction for orthodontic reasons were obtained after informed consent and in accordance with the local ethical committee (Ethikkommission beider Basel, Ref. Nr. 01/10 and 232/10). Mature, healthy, one- or two-rooted premolars were removed by oral surgeons under minimal trauma and without levers and visually inspected for the intactness of the residual PDL and the root surface (Tsukiboshi 2002). Teeth were immediately transferred to commercial storage solutions (DentoSafe®, Medice, Iserlohn, Germany; SOS-Dentobox®, Miradent, Duisburg, Germany, or Curasafe®, Curaden, Kriens, Switzerland), and processed for cultivation within 24h.

Root slices were prepared essentially as described (Preisig & Schroeder 1988; Boll 1994; Pohl et al. 1999). Teeth were seized at the crown with extraction forceps (Ustomed, Tuttlingen, Deutschland) without touching the root and rinsed with phosphate-buffered saline (PBS; pH 7.4). Forceps were fixed in

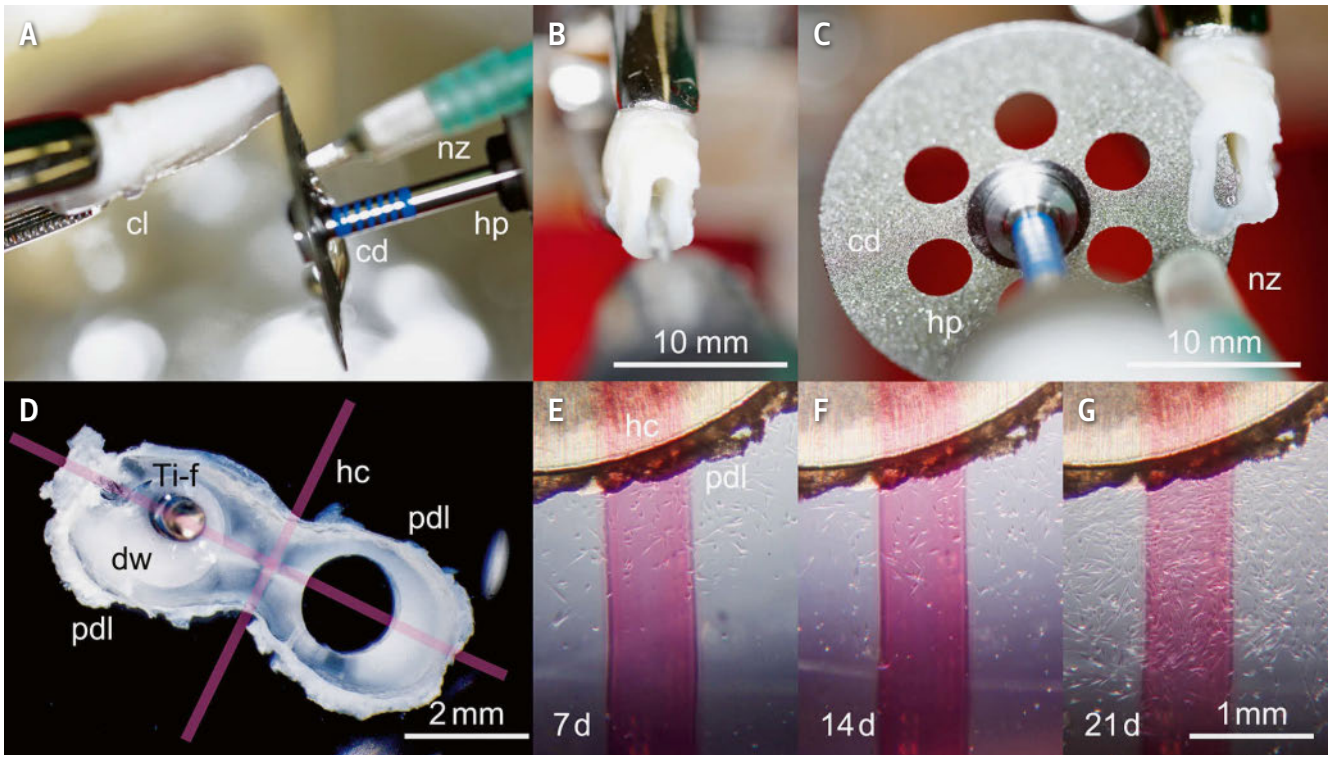

Fig. 1 Preparation of root slice cultures.
A) Separation of apex from freshly extracted tooth: clamp (cl), nozzle with 4 °C sterile saline (nz), cutting disk (cd) in handpiece (hp). B) Broadening of root channel after pulp removal. C) Slicing. D) Slice immobilisation: residual periodontal ligament (pdl), Ti-file fused into well bottom (Ti-f), dental wax (dw), cross-lines (hc) on the underside of the culture well. E–G) Cell outgrowth and expansion: after 15 (E), 22 (F) and 29 days (G) in culture, or one, two or three weeks upon cell outgrowth, respectively.

a clamp holder, where slicing and abrasion was performed under a continuous jet of sterile saline (150 mM) from a 1,000 ml infusion bottle precooled to 4 °C. Apex (3 mm) and slices were cut with a diamond-coated separating disc (Brasseler-Komet, Lemgo, Germany), abrading of the pulp chamber was done with a bur (1.6 mm Ø Retropost®-drill, Brasseler-Komet, Lemgo, Germany). Both were fixed in a dental handpiece and operated either mounted on a sliding device (Boll 1994) or by hand. After apectomy, the pulpa was removed by forceps and the adjacent dentin abraded, by broadening the root canal with a bur, from retrograde. Depending on their length, roots were segmented into 6 to 9 about 1 mm thick slices (Fig. 1A–C).

Immediately after separation, each slice was washed in 500 µl PBS and temporarily stored in 500 µl cell culture medium (Dulbecco's minimal essential medium DMEM, complemented with 1% Penicillin/1% Streptomycin [5,000 IU/ml P, 5,000 µg/ml S], 1% L-Glutamine [200 mM], 1% Na-Pyruvate [100 mM] all from GIBCO®, and with 10% Fetal Bovine Serum [FBS; OXOID, ThermoScientific, UK]) until (≤ 5 min) transfer to the culture plate.

Slice cultures were done in 24-well cell culture plates (Falcon®). In advance, plates had been fitted under sterile conditions with 3 to 4 mm bits of Ni-Ti-K-files (K-File NITIFLEX®, ISO-sizes 30–60, Maillefer, Oklahoma, USA), which were heated with a Bunsen and pressed to the centre of each culture well to fuse into the bottom. Root slices were placed over these pins, immobilised with a drop of dental wax (modelling wax "SUPERIOR" pink, Belladi Ruscher, Altnau, Switzerland), and then covered with 300 µl cell culture medium. The slice area was divided into four quadrants by a cross-line drawn onto the well's underside (Fig. 1D). Culture plates were kept in an incubator (Heraeus Instruments, Hanau, Germany) at 37 °C under an atmosphere of 5% CO_2.

Slice monitoring by visual inspection was done daily, on weekends every other day, under an inverse microscope (Leitz DM IL, Leica, Solms, Germany) with an eyepiece equipped with a 1 mm^2 calibrated grid divided into 100 µm × 100 µm squares. Cell types were not specified. Instead cell growth was related to that of adherent PDL fibroblast-like cells (PDF) as an indicator for overall growth.

Initiation of cell outgrowth (ICO) was registered for each slice as the days in culture until first PDF(s) were noted.

Vitality of a slice refers to the presence of at least one PDF. Hence, a vital tooth displays at least one vital slice, and a vital slice at least one vital quadrant.

Expansion of outgrown cells (EOC) was measured at day 20 in each vital quadrant as the perpendicular distance between growth front and root surface measured by the calibrated grid in 100 µm units. Partially covered squares were rated as full units; outgrowth beyond 900 µm was rated as 1 mm.

ECO_{20} corresponds to the measured EOCs averaged over the vital quadrants.

Circular growth (CRG), i.e. the distribution of cells around a slice at day 20, was noted for each slice as the number of vital quadrants, and expressed as polar coordinates: all quadrants vital: 360°, CRG = 1; three quadrants: 270°, 0.75; two quadrants: 180°, 0.50; one quadrant: 90°, 0.25. In a rough estimate the polar coordinates would correspond to the vital fraction of a slice's relative mantle surface.

CRG_{20}, the circular growth at day 20 around the root axis, was expressed as the sum of the CRGs averaged over the vital slices.

Axial density (AD_{20}) was calculated as the vital fraction of a tooth's total (vital + nonvital) slices having remained in culture until day 20.

Selection criteria for serial-cut slices representing single teeth: teeth were cultured on availability, i.e. on a rolling basis. For exploring the practicability of representing a tooth's residual PDL in culture by serial-cut slice series, the following requirements were fixed empirically in advance: slice series were cultured at least until day 20. Slices displaying in culture mechanical damage or infection were discarded immediately. All slice series were evaluated post hoc for representativeness. Series qualifying as representative for a teeth had to include at day 20 at least two slices from its apical (A), mesial (M), and cervical (C) root zones in culture. Note: since slice series vary between 6 and 9, depending on a root's length, slices had been attributed to root zones as follows: tooth T cut in 9 slices: T9: 3A, 3M, 3C slices; T8: 3, 2, 3; T7: 2, 3, 2; T6: 2, 2, 2. The final study was to include at least ten teeth matching these inclusion criteria.

Out of 19 processed teeth (140 slices), 11 teeth (57.90%), represented by 81 slices (57.86%) had complied with the inclusion criteria post hoc. Five teeth were one-rooted, six two-rooted; five were from female, six from male donors. The average donor's age was 14 ± 1.5 years (range 11.96–16.49). Slices were 0.83 ± 0.22 mm thick. In two rooted teeth, only the root with more evaluable slices was considered.

Results

Noninvasive monitoring over time

The benefit of slice immobilisation is illustrated by the micrographs in Figure 1 (E–G) displaying slice 6 of tooth 3 (Fig. 3) after 16 (F), 23 (G), and 30 (H) days in culture or 7, 14 and 21 days after ICO, respectively. Even if the culture plate had to be moved daily for monitoring or for changing media, the position of the residual PDL had remained unchanged with respect to the red cross-line (Fig. 1 F–H).

In addition to visual inspection, on a trial base the situations at ICO, and four and five days later, were documented at the same site by phase contrast images (Fig. 2) for a single slice (Fig. 2 tooth 3, slice 6). At ICO (Fig. 2A) few PDFs, obviously advanced out of the PDL edge onto the well bottom, are surrounded by buoyant debris and spherical cell-like and subcellular structures, together with two aggregate-like ruffled spheres. Four days later (Fig. 2B) the PDL-derived cell population has markedly increased. PDF's are majorly spread onto the well bottom. Some cells display long spindle-like or fusiform shapes. After another day (Fig. 2C), cells have increased in number and changed in space. Moreover, the fraction of the spread-out PDFs seems to have diminished in favour of long spindle-shaped and fusiform cells and of poorly or nonadhering roundish structures.

Initiation of cell outgrowth (ICO)

Figure 3 displays for the individual teeth and their slices the number of days slices had remained in culture until ICO. The vertical bars symbolise the root axes; the horizontal bars the vital slices, and their lengths the days in culture until ICO. Bars are stacked along the root axes in the sequence of slicing (bottom: apex), nonvital slices manifest as spaces.

The earliest outgrowth was perceived after two days in culture (Fig. 3, tooth 3 and 4). Of the 81 slices cultivated, 55 slices (67%) from all 11 teeth had shown outgrowth, i.e. were vital. Within the first week of incubation, 64%, and within the first two weeks, 96% of the vital slices had shown ICO. On average, ICO was perceived after 7.3 ± 4 days.

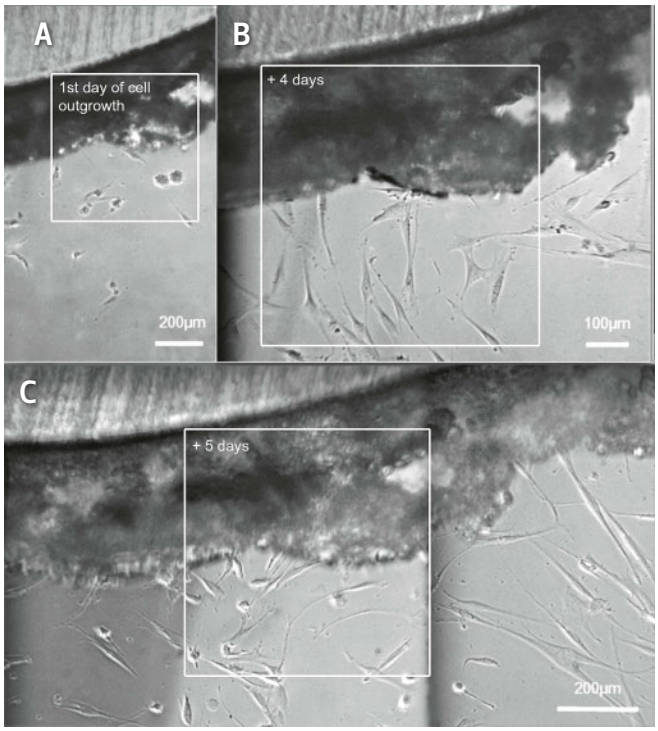

Fig. 2 Cells growing in vitro out from the residual periodontal ligament over time
Phase contrast images of a root slice in culture displaying the site of the initiation of cells growing out (ICO) from the residual periodontal ligament (PDL): at ICO (A: top left) and four (B: top right) and five days later (C: bottom). Note: In this exploratory phase, cell types were not characterised. The day of the first outgrowth was related to that of the first appearance of PDL fibroblast-like cells, which served as a reference. Images were taken on a trial base at the site of ICO of a single, randomly selected slice. They provide anecdotal evidence.

Expansion of outgrown cells (EOC), axial density (AD), circular growth (CRG)

The average distance between root surface and growth front at day 20, EOC_{20}, is displayed for the individual slices of the respective teeth as horizontal bars stacked in the sequence of slicing (bottom: apex). Nonvital slices manifest as spaces (Fig. 4).

The axial densities (AD_{20}) describing the relative fraction of vital slices along the root axes are evident in Figures 3 and 4. Averaged over all teeth, AD_{20} amounted to 67.29%.

The pie diagrams below each profile represent the respective average of vital quadrants around the root axes and correspond to the fraction of the root circumference lined by vital PDL (CRG_{20}), which relates to the average of the CRGs of a tooth's slices.

In short, the profiles in Figure 3 display for each individual tooth and its slices the ICO, while those in Figure 4 represent i) the relative distribution of vital and nonvital slices along the root axes (AD_{20}), ii) the average EOC of the vital slices in mm, and iii) the average fraction of vital PDL cells growing around the root surface (CRG_{20}).

On average, ICO initiated after 7.3 ± 4 (sd: standard deviation) days in culture relating to a coefficient of variation (cv) of 30%. The eleven teeth displayed an average AD_{20} of 67 ± 29 (sd)% (cv: ± 43%), an EOC_{20} of 305 ± 127 (sd) µm (cv: ± 42%) and a CRG_{20} of 63 ± 32 (sd)% (cv: ± 51%).

Discussion

The immediate reinsertion of healthy, freshly extracted teeth with the tooth-sided part of the ruptured PDL still attached, has been reported time and again as highly successful (Andreasen et al. 1990; Cross et al. 2013; Yoshino et al. 2013). By removing apex and pulp tissue from such teeth and cultivating the roots as series of consecutively cut, transversal sections, such pulp-free slices may retain the properties of the residual PDL to propagating successful tooth reintegration, and unfold the "innate potential of the periodontal tissue in providing meaningful periodontal regeneration" (Lin et al. 2015) in vitro, as do freshly extracted teeth with intact root surfaces in vivo.

Pulp-free root slices had been cut for some time from extracted and enzymatically debrided or planed and/or demineralised teeth after pulp removal. They served as substitutes for denuded root surfaces, or pair-wise, for periodontal space when modelling the reattachment of gingiva or PDL in vitro with the respective cell preparations (Pitaru et al. 1983; Preisig & Schröder 1988; Aukhil et al. 1990). More recently, the walls of the pulp channel of such slices were used as scaffolds in conjunction with pulp regeneration (Sakai et al. 2011). Cultures of nondenuded pulp-free root slices had served as a primary source for PDL-derived cells when investigating PDL cell attachment to denuded autologous root surfaces (Preisig & Schröder 1988), or as indicators when testing various storage solutions for avulsed teeth (Boll 1994; Pohl et al. 1999). Nonetheless, still today the most common sources for expanded PDL-derived cell populations are biopsies from periodontal soft tissue adhering to extracted roots, or enzyme-debrided cells or mechanical scrapings from the root surface (Arnold & Baram 1972; Piche et al. 1989; Marchesan et al. 2012; Tran et al. 2014; Arnold et al. 2010; Avinash et al. 2017).

Since in vitro cultivation and noninvasive cell monitoring of whole teeth over time is not trivial, we explored instead the possibility of representing an extracted tooth's root surface with its residual PDL in culture as a series of its consecutive slices comprising the whole root surface with its apical, mesial and cervical zones. Accordingly, apex and pulp were removed and the pulp chamber abraded as described by Preisig & Schröder (1988). The slicing conditions and those for intermittent storing during processing were essentially those elaborated by Boll (1994) and Pohl et al. (1999). In contrast to Pitaru et al. (1983) slices were not glued to the bottom of the culture well. They were just inserted over an aforemounted vertical pin, stabilised with wax, covered with medium and incubated.

As a first test of relevance, the days in culture until ICO i.e. the outgrowth dynamics of PDL cells in vitro, were compared to the time course of cell outgrowth at the root surface in vivo, as delineated by histology upon reinsertion of extracted teeth (Temmerman 2006; Sato et al. 2010; Panzarini et al. 2013; Dickinson et al. 2013; Haemmerle & Giovannobile 2014).

At this exploratory stage, individual cells were not characterised. ICO was simply related to the emergence of PDFs, i.e. periodontal ligament fibroblast-like cells. Such adherent cells are common in PDL explant cultures where they make up the largest part of the population (Lekic & McCulloch 1996; Piche et al. 1989). In contrast to simply basing ICO on the emergence of the rather broadly defined PDFs, the proper characterisation of outgrowing cells could easily turn out as disproportionate for the exploratory phase: 'fibroblast' generally refers to stromal cells that do not express lineage-specific markers, such as smooth muscle cells, pericytes or multipotent progenitors (Hinz 2013), which can render the characterisation of outgrowing PDL cells rather complex (Marchesan et al. 2011; Avinash et al. 2017). Moreover, upon augmentation of PDL-derived cells by multiple passaging, cells with fibroblast-like morphology may loose specific markers already in the first passages (Itaya et al. 2009), and with increasing passage number, e.g. fibroblasts and MSCs, will become phenotypically indistinguishable (Denu et al. 2017).

First outgrowing cells (Fig. 3) were noted in culture after 2 days. After the first week the majority, and after the second week essentially all vital slices had shown ICO. Such dynamics agree with the time course delineated for tooth-sided periodontal regeneration upon tooth extraction and reinsertion in vivo, i.e. a rapid increase of ICO in the early (2–5 days) and extending to the intermediate phase (9–14 days), to then level off for the regeneration phase (Temmerman 2006; Sato et al. 2010; Panzarini et al. 2013; Dickinson et al. 2013; Haemmerle & Giannobile 2014; Sculean et al. 2015). Accordingly, the 20 days of monitoring, set empirically for technical convenience, proved adequate for following in vitro the early and intermediate events relating to tooth-sided periodontal reintegration elapsing in vivo.

The distribution of the residual PDL varied considerably along and around the root axes within and among individual teeth (Fig. 4). Nonetheless, the average AD_{20} of 67 ± 29% measured at

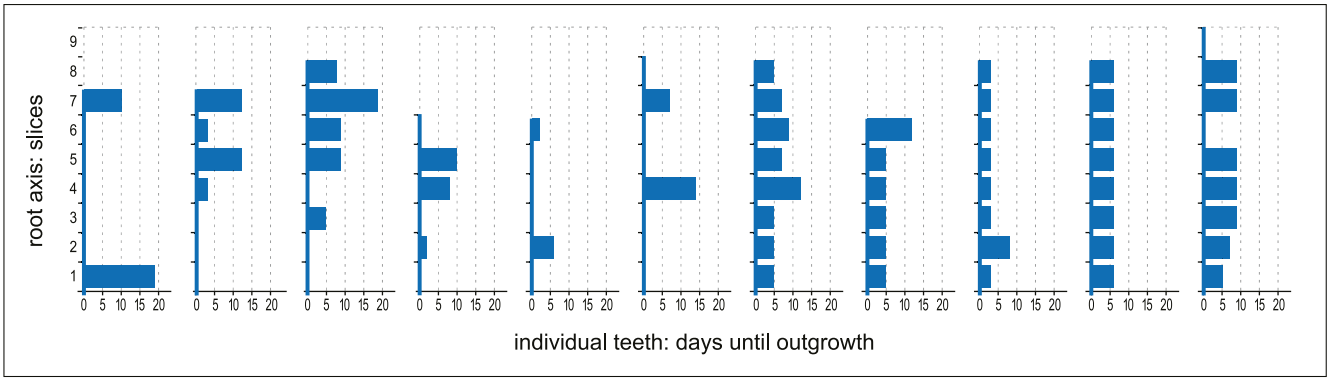

Fig. 3 Days in culture until initiation of first cell outgrowth (ICO)
Vertical bars represent the root axes of the individual teeth. The horizontal bars stacked in the sequence of slicing (bottom: apex), indicate their vital slices, their lengths correspond to the days in culture until perception of outgrowing periodontal ligament fibroblast-like cells (PDFs). Nonvital slices manifest as empty spaces.

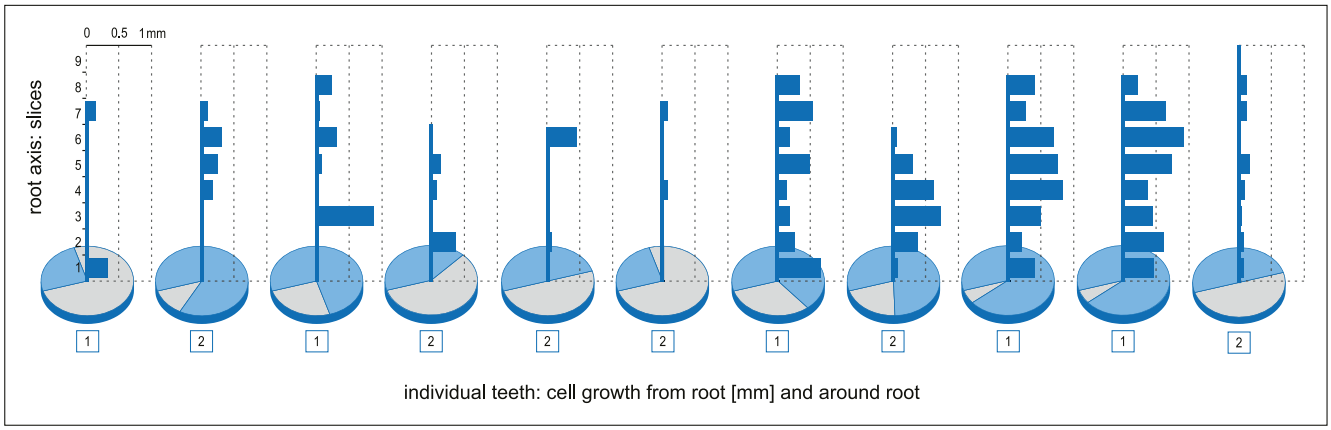

Fig. 4 Distribution of PDL fibroblasts on the root surface in culture at day 20.
Expansion of outgrowing cells from the root surface and distribution of vital PDFs along and around the root axes: horizontal bars along the root axes represent the vital slices of individual teeth stacked in the sequence of slicing (apical: bottom). Their length represents the distance between root surface and growth front measured in mm and averaged over the vital quadrants. Nonvital slices manifest as spaces.
The disc at the base of each profile represents the distribution of vital tissue around a tooth's root axis, i.e. the average of vital quadrants over a tooth's vital slices. The numbers 1 or 2 indicate the rootedness of each tooth.

20 days in culture seems plausible, when compared to the reported 55.4 ± 22.5% of PDL, having remained along the root axes immediately after extraction (Shimono et al. 2003). Even if such variations may appear substantial, it may be noteworthy, that distribution and vitality of the residual PDL do not only depend on technical parameters such as the mode of tooth removal or on extraoral exposure (Andreasen & Kristersson 1981). Being donor-specific, they depend also on donor site, donor age and oral health (Haas et al. 2008), which are further adding to the variability. For putting such variations in better evidence, the values for ICO, and for EOC_{20}, AD_{20} and CRG_{20} were displayed individually, for each slice within its proper tooth (Fig. 3 and 4). At first sight, the homogeneity among the individual teeth with respect to ICO seems rather poor (Fig. 3). In the exploratory phase, this could be an indication for lacking experimental routine, though, also mere coincidence cannot be excluded. Moreover, some profiles indicate that ICO within, but not between single teeth, seems to proceed in synchrony, i.e. a situation attributable to the extraction trauma, or – conceivably – also to slicing.

Though, in this exploratory study, a characterisation of PDL-derived cells was not intended, we did not want to withhold some images that provide anecdotal evidence of the population of cells growing out of the residual PDL changing early on over time in number, shape and space. The snapshots taken on a trial base in parallel to visual monitoring show for a single slice the same site of the residual PDL at ICO as well as four and five days later (Fig. 2). By and large, at ICO two aggregate-like ruffled spheres show in between two distinct adherent PDFs, i.e. our marker cells for ICO. They are surrounded by buoyant debris and cell-like and subcellular roundish structures. Arnold et al. (2010) identified similar aggregate-like spheres as stem cell aggregates early on in periodontium-derived stem cell culture. After four and five days the outgrowing cells had increased and expanded and changed into a heterogenic cell population. A cell population, changing during the early phase of repair in number and heterogeneity of shape in space over time, agrees with the presence of PDFs, long spindle-shaped and fusiform stem/progenitor cells, and of round cell-like and subcellular structures, etc., as documented or even characterised in various PDL explant cultures, often wide before confluence (Piche et al. 1989; Arnold et al. 2010; Archana et al. 2014; Tran et al. 2014; Avinash et al. 2017) and complies with the repair processes initiated in mesenchymal tissue upon acute mechanical injury (Weiss 1961; Dickinson et al. 2013; Greaves et al. 2013).

In accordance with our hypothesis, ICO from the residual PDL lining the surface of root slices in culture seems to follow the dynamics of cell growth on the root surface of reinserted teeth in vivo. Based on these exploratory results, we conclude that it will be worthwhile to verify our findings, i.e. to further elaborate the model by fine-tuning preparation techniques and timing, and to characterise and identify outgrowing cells in relevant sample sizes and in conjunction with proper statistics. Beyond that, documenting noninvasively the cells emerging out of the residual PDL seems to be feasible. There is a fair probability that even in our still simple set-up, cells emerging out of the residual PDL and putatively differentiating over time may be followed systematically, and documented and characterised over time.

Complementing such tooth-sided slice cultures – possibly at various differentiation stages – with the various cytokines engaged in periodontal regeneration (Morand et al. 2016), or with other tissues or matrices such as the residual PDL remaining at the alveolar bone of the extraction socket (Sculean et al. 2015), with appropriately engineered constructs or cell free scaffolds (Farag et al. 2014; Bartold et al. 2016) such as bone, dentin or biomimetic or xenobiotic surfaces (Ripamonti et al. 2011; Green et al. 2016) or by modulating its microenvironment (Yamamoto et al. 2018), may contribute to eventually emulating the integration process of healthy and possibly also of compromised teeth in vitro. Concomitantly, the model may also facilitate to gain further insight into the cascade of events elapsing during general mesenchymal tissue repair triggered by acute mechanical injury (Weiss 1961) at a cellular and subcellular level.

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Zusammenfassung

Einleitung

Die Wiedereingliederung frisch extrahierter gesunder Zähne ist sehr erfolgreich, höchstwahrscheinlich wegen der Regenerationsfähigkeit ihres «residual periodontal ligaments (PDL)», d.h. ihres auf der Wurzeloberfläche verbleibenden Restdesmodontes. Wir stellten deshalb die Hypothese auf, dass in Kultur, die einzelnen Scheiben einer segmentierten Zahnwurzel die Fähigkeit haben, aus ihrem Restdesmodont die Population von PDL-Zellen freizusetzen, die sich zahnseitig an der Reintegration eines Zahnes beteiligten.

Material und Methoden

Zur Prüfung dieser Hypothese wurden Apex und Pulpa humaner Prämolaren entfernt und deren Wurzeln in 6 bis 9 etwa 1 mm dicke Scheiben aufgetrennt. Jede Pulpa-freie Scheibe eines Zahnes wurde in ihrem Kulturgefäss einzeln immobilisiert, für mindestens 20 Tage bebrütet, und unter täglicher Kontrolle auf Präsenz erster auswachsender Zellen (PEAZ) inspiziert. Die Immobilisierung der Scheiben ermöglichte, die Kulturen nicht invasiv über die Zeit zu beobachten. Die Tage in Kultur bis zur PEAZ wurden für jede Scheibe registriert. PEAZ wurde auf das Vorhandensein der am häufigsten vorkommenden PDL- Fibroblasten-ähnlichen Zellen (PDF) bezogen. Die auswachsenden Zellen wurden nicht weiter charakterisiert. Nach 20 Tagen in Kultur wurde die Verteilung der auswachsenden Zellen auf der Wurzelscheibe und die Distanz zwischen der Wachstumsfront auswachsender Zellen und der Wurzeloberfläche registriert, ebenso wurde die Verteilung vitaler Scheiben entlang und die zirkuläre Verteilung der Zellen um die Wurzelachse des Zahnes notiert.

Ergebnisse

Von den 81 Scheiben der 11 Zähne zeigten 55 PEAZ, 64% innerhalb von einer Woche und 96% innerhalb von zwei Wochen. Die Dynamik des Auftretens erster Zellen in vitro war mit der mittels Histologie bestimmten frühen (Tag 2–5) und intermediären (Tag 9–14) Integrationsphase vergleichbar.

In einer ersten Versuchsserie zeigten bei PEAZ aufgenommene Bilder einer einzelnen Scheibe wenige PDF, Zelltrümmer und zellartige und subzelluläre kugelige Gebilde sowie Zellaggregat-artige Strukturen. Nach vier Tagen hatten hatte sich an derselben Stelle die Zellpopulation deutlich vermehrt und bestand jetzt vor allem aus adhärenten weit ausgespreizten PDF. Innerhalb eines weiteren Tages vermehrte sich die Population etwas weiter. Die Zellen präsentierten sich allerdings als ein Gemisch von adhärierenden, vorwiegend ausgestreckten und fusiformen PDF und von runden, zellartigen Strukturen.

PEAZ und die Verteilung der vitalen Scheiben entlang, das Zellwachstum um und die Expansion der herauswachsenden Zellen von den Wurzelachsen weg nach 20 Tagen wurden für die einzelnen Zähne als 3-D-ähnliche Profile angezeigt.

Diskussion

Die Ergebnisse dieser ersten exploratorischen Studie deuten darauf hin, dass es möglich ist, die Zellaktivitäten im Restdesmodont auf Wurzelscheiben in Kultur zu erhalten. Im Einklang mit unserer Hypothese scheinen sich dessen Zellen in vitro ähnlich zu verhalten wie bei der zahnseitigen Reintegration in vivo. Die Relevanz und die Robustheit des Modells soll nun in einer entsprechend umfangreicheren Studie untersucht werden.

Die wenigen Fotos, die bei PAEZ und einige Tage später eine Zellpopulation aufzeigen, die sich im Laufe der Zeit in Anzahl, Form und im Raum verändert, illustrieren nur einen einzigen PDL-Abschnitt einer zufällig ausgewählten Scheibe, was die Bilder als anekdotische Evidenz wertet. Allerdings zeigen sich an der einen Stelle im zeitlichen Verlauf Zellmorphologien, wie sie für mehrere PDL-Explantkulturen als Stamm-/Progenitorzellen beschrieben wurden.

Prinzipiell sollten es immobilisierte Wurzelscheiben in Kultur schon jetzt erlauben, die Zellproliferation und/oder die Differenzierung einzelner Zellen über die Zeit bzw. in Wechselwirkungen mit benachbarten Strukturen nicht invasiv zu verfolgen. Letztendlich könnte dieses System die Identifizierung von reintegrationsbedingten zellulären Veränderungen, wie sie durch mechanisches Trauma ausgelöst werden, erleichtern und es ermöglichen, zelluläre Wechselwirkungen z.B. zwischen der Wurzeloberfläche und dem Alveolarknochen oder zwischen natürlichen oder künstlichen Gerüsten in einem In-vitro-Modell in einer In-vivo-ähnlichen Situation zu studieren.

Résumé

Introduction

La réintégration des dents saines et fraîchement extraites est très réussie, probablement en raison de la capacité de régénération du ligament parodontal résiduel (PDL). Nous avons émis l'hypothèse qu'une racine de dent extraite, quand coupée et cultivée en tant que tranches consécutives, représentera l'entité des cellules du PDL engagées dans la réintégration des dents saines.

Matériel et méthodes

Pour confirmation, les apex et la pulpe ont été enlevées des prémolaires humaines. Leurs racines ont été séparées en 6–9 tranches d'environ 1 mm d'épaisseur. Chaque tranche dépulpée a été immobilisée dans son puits de culture. Les tranches ont été incubées pendant au moins 20 jours et inspectées quotidiennement pour l'initiation de l'excroissance des cellules (IEC). L'immobilisation de tranches facilitera de surveiller les sites d'IEC de manière non invasive en course de temps. Les jours en culture jusqu'à l'IEC ont donc été registrées pendant une période de 20 jours de culture. A ce temps là, l'avancement des cellules PDL sortant de la bordure du PDL a été mesurée, tout comme la distribution circulaire des cellules autour de l'axe des racines. Dans cette phase exploratoire, les cellules dérivées de la PDL n'ont pas été caractérisées davantage. La dynamique des cellules était juste liée à celle des cellules de type PDL-fibroblaste, qui sont les plus abondantes dans les cultures primaires de PDL explanté.

Résultats

Parmi les 81 tranches de 11 dents, 55 ont montrées IEC, dont 64% dans la 1re et 96% en deux semaines. Leur dynamique était comparable à celle des cellules PDL in vivo dans la phase d'intégration précoce (jour 2–5) et intermédiaire (jours 9–14) déterminées par l'histologie.

Sur une base d'essai, une première série de micrographies documentait une seule coupe en course de temps démontrant à la site d l'ICE peu de cellules type-fibroblastes (PDF) et de type putatif cellules souche, qui sortaient de la bordure du PDL résiduelle, entourées des débris cellulaires et des particules cellulaires et subcellulaires peut adhérentes. Au bout de quatre jours, la population cellulaire du même site avait changée en population majoritairement de PDF adhérents et étendus. Après un autre jour elle semblait d'avoir assumées des formes plutôt plus allongés et fusiformes et des structures rondes peu adhérentes.

L'IEC et la distribution des tranches vitales le long, la croissance cellulaire autour et l'expansion des cellules à l'écart après 20 jours ont été représentées par des profiles 3-D naïfs pour chaque dent et ses tranches de racines cultivées.

Discussion

Cette étude exploratoire indique qu'en culture de section de racine, les cellules PDL se comportent de manière semblable à celles du PDL qui s'engagent dans l'intégration des dents replantées in vivo. Après cette phase exploratrice, qui s'est montrée en faveur de notre hypothèse, la praticabilité du modèle est à confirmer dans une étude appropriée et plus étendue. Notez que les quelques photographies représentant sur le site de l'IEC une population cellulaire en changeant en nombre, forme et en espace au fil du temps concernent juste un seul site d'une seule tranche choisie au hasard. Elles devraient être considérées comme évidence anecdotique.

Néanmoins, ces images démontrent des structures similaires aux cellules souche/progénitrices comme déjà identifié in vivo et dans diverses cultures PDL-explants, surtout à l'initiation de l'élevage.

En principe, un tel modèle devrait permettre un suivi non invasif des cellules individuelles au fil de temps et au cours de leur prolifération et différenciation, éventuellement à partir de l'excroissance cellulaire déclenchée par le traumatisme immédiat par l'extraction ou – au moins concevable – par le coupage de la racine, pour initier la réparation tissulaire. Par ailleurs, le modèle permettrait de tracer les événements cellulaires et leurs interactions avec les structures avoisinantes comme l'os alvéolaire, mais aussi avec des constructions d'ingénierie tissulaire ou des échafaudages naturels ou artificiels.

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