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Review Article

Dental Pulp Stem Cells as a Cell Source for Craniofacial Regeneration

Zupancic A^{1*}, Fabbri R² and Pichler V³

¹Department of Haematology and Stem Cell Transplantation, University of Maribor, Maribor, Slovenia

²Department of Haematology and Stem Cell Transplantation, Sapienza University of Rome, Rome, Italy

³Department of Clinical Immunology, University of Innsbruck, Innsbruck, Austria

***Address for Correspondence:** Ales Zupancic, Department of Haematology and Stem Cell Transplantation, University of Maribor, Maribor, Slovenia; E-mail: ales.zupancic@universityofmaribor.edu

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Abstract

Background: Dental pulp is routinely discarded with extracted teeth and yields clonogenic multipotent cells ontogenetically native to the craniofacial skeleton, making it an attractive graft source.

Objective: To separate what the laboratory and clinical literature measures from what it asserts, and to state the size and design of the human craniofacial evidence.

Methods: Narrative review of characterisation studies, human case series, randomised trials and banking reports.

Findings: Pulp cells formed 22 to 70 colonies per 10,000 cells against 2.4 to 3.1 for marrow stromal cells. Craniofacial bone experience is a seven-patient split-mouth cohort reported twice, with probing depth gain 6.3 ± 2.1 mm against 4.5 ± 1.4 mm ($p < 0.001$, $N = 7$), and a six-patient cleft series.

Conclusions: The evidence supports dental pulp cells as an accessible, well-characterised source that makes mineralised matrix. It does not yet support clinical translation, which needs a controlled craniofacial bone trial.

Keywords: Dental pulp stem cells; Craniofacial regeneration; Alveolar bone grafting; Regenerative endodontics; Cell banking

Introduction



Dental pulp is the only mesenchymal tissue in the body that patients routinely discard. Third molars, orthodontically extracted premolars and exfoliating deciduous teeth are removed by the million each year, and each carries a small vascularised soft-tissue core from which clonogenic, multipotent cells can be recovered. That logistical fact, more than any measured biological superiority, is why dental pulp stem cells have been pursued as a craniofacial graft source since their first isolation [1].

The case made for them in the literature runs roughly as follows: the cells are neural-crest-derived and therefore ontogenetically matched to the jaws and midface; they are more clonogenic and more proliferative than bone marrow stromal cells; they make dentine, bone, endothelium and neuron-like cells; and they can be banked from a tissue that costs the patient nothing. Each of those claims has a different evidential weight, and reviews of this field have tended to flatten them into a single enthusiastic register. Two errors recur often enough to be worth naming at the outset. The seven-patient mandibular series is regularly counted twice, once as d'Aquino's three-year-old report and once as Giuliani's three-year follow-up, which doubles the apparent craniofacial bone evidence [2,3]. And the one properly randomised, controlled trial of cell-based regenerative endodontics is repeatedly cited as dental pulp evidence when its cells were umbilical-cord mesenchymal stromal cells [4].

This review separates what is measured from what is asserted. Laboratory characterisation is treated as strong; the human craniofacial bone literature is treated as what it is, a small number of uncontrolled or historically controlled series; and where the field's most-repeated numbers could not be traced to a primary source, no number is printed.

Origins and defining properties of dental pulp stem

cells

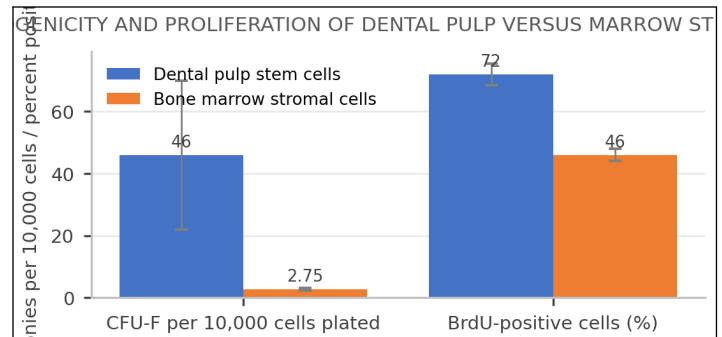


Figure 1: Colony-forming efficiency (plotted as the midpoint of the reported 22-70 and 2.4-3.1 ranges, bars spanning the range) and BrdU incorporation (mean, SEM, N = 3 per group) for dental pulp stem cells against bone marrow stromal cells in a single study of donors aged 19-29 years with commercially sourced, non-donor-matched marrow [1].

The dental papilla derives from cranial neural crest, and lineage tracing in mice established that crest cells populate the tooth mesenchyme and the mandibular skeleton from the same migratory stream [5]. Pulp stem cells therefore share a developmental address with the bones they are proposed to repair, which is the field's most defensible mechanistic argument and its least quantified.

Gronthos and colleagues isolated adherent clonogenic cells from adult third-molar pulp, expanded them, and showed that transplanted with hydroxyapatite/tricalcium phosphate they generated a dentine-like matrix lined by human odontoblast-like cells around a vascular pulp-like interstitium [1]. Colony-forming efficiency was 22 to 70 colony-forming units per 10,000 nucleated cells plated, against 2.4 to 3.1 per 10,000 for bone marrow stromal cells; donors were aged 19 to 29 years and the marrow comparator was commercially sourced rather than donor-matched, and the paper does not state how many donors or teeth contributed. Follow-up work confirmed the cells sit in a perivascular niche shared with marrow stromal cells [6], express embryonic and mesenchymal markers in various combinations, and meet the ISCT minimal criteria for multipotent mesenchymal stromal

cells [7] — criteria that were written for marrow and adipose populations and that dental populations satisfy without thereby being equivalent to them.

Related populations were characterised in quick succession: stem cells from human exfoliated deciduous teeth [8], periodontal ligament stem cells [9], and stem cells from the apical papilla of immature permanent teeth [10]. The apical papilla population supported the swine bioroot experiments that remain the most complete demonstration of engineered tooth-supporting structure in a large animal [11]. A separate Italian line of work described a stromal bone-producing subset that formed woven bone in vitro and, on transplantation, bone containing entrapped osteocytes together with human-derived vessels, arguing that osteogenic and endothelial differentiation occur in parallel from the same starting population [12,13].

What the comparative bench data actually support

Two comparative numbers from the founding study carry most of the field's weight. Colony-forming efficiency, given above, and proliferation measured by bromodeoxyuridine incorporation: 72% ± 3.48 (SEM) of pulp cells were BrdU-positive against 46% ± 1.96 for marrow stromal cells, with three replicates per group [1]. Three replicates, one laboratory, unmatched donors. This is a real difference and a thin dataset, and it has been repeated for twenty-five years without a donor-matched replication being widely cited in its place.

Lineage capacity is better supported than proliferative superiority. Twelve single-colony-derived strains were assayed for odontogenic potential; two-thirds generated abundant ectopic dentine in vivo and the remaining third only a limited amount, which establishes both multipotency and clonal heterogeneity within a single pulp [14]. Side-by-side transplantation showed that pulp-derived and marrow-derived populations produce structurally distinct tissues — dentine-pulp complex

against lamellar bone with haematopoietic marrow — rather than one being a better version of the other [15]. Donor-matched comparisons in rodents have since been performed [16], and the biology of dental versus non-dental mesenchymal populations has been reviewed at length [17], but a single authoritative donor-matched bone-formation figure is not available to quote.

Table 1: Dental pulp stem cells and deciduous pulp cells against bone marrow stromal cells on measures traceable to a primary source; deciduous-pulp entries are left as not confirmed rather than filled from secondary citation [1,14,15,33,7,8].			
Measure	Dental pulp	Deciduous pulp	Bone marrow
CFU-F per 10,000 cells plated	22-70	not confirmed	2.4-3.1
BrdU-positive cells (% , SEM, N = 3)	72 (3.48)	not confirmed	46 (1.96)
Clonal strains giving abundant ectopic dentine	two-thirds of 12 strains	not confirmed	forms bone and marrow, not dentine
Cultures recoverable from extracted teeth	31 of 40 (77.5%)	not confirmed	not applicable
ISCT minimal stromal criteria met	yes	yes	yes

Deciduous pulp cells differentiate into functional odontoblasts and endothelium and, seeded into emptied root canals in immunodeficient mice, regenerate a vascularised pulp-like tissue [18,19]. Pulp cells also acquire voltage-gated sodium currents and fire action potentials under appropriate cues, consistent with their crest origin [20]. What the bench literature does not supply, for the deciduous population in particular, is a verifiable proliferation figure: the claim that these cells outgrow both adult pulp and marrow cells appears qualitatively in the original report but no quantitative version of it could be confirmed at source, and none is printed here.

The practical consequence is that the bench literature justifies using these cells, not preferring them. Nothing in the comparative data establishes that a pulp-derived graft outperforms a marrow-derived one in a jaw defect; what it

establishes is that a pulp-derived graft is obtainable without a second surgical site, from tissue already destined for disposal, in a population of cells that will expand readily and will make mineralised matrix when asked. For a craniofacial indication that is a sufficient rationale on its own, provided the clinical claims are scaled to it.

Craniofacial bone: the human series

The craniofacial bone evidence consists of two series and fewer than fifteen patients in total.

The mandibular work is a split-mouth design in seven patients, each contributing a test site grafted with autologous pulp cells on a collagen sponge and a control site, with histomorphometry read blind by two observers [2]. At three years, probing depth gain was 6.3 ± 2.1 mm at test sites against 4.5 ± 1.4 mm at control sites ($p < 0.001$, $N = 7$), and the regenerated tissue was compact lamellar bone with Haversian systems rather than the spongy bone the defect would normally fill with [3]. The mineral density result runs the other way from how it is usually cited: the high-mineral-density fraction was $57.7 \pm 15.0\%$ at treated sites against $69.3 \pm 6.7\%$ at the patients' own control sites. Drilling resistance was 36 N·cm at test sites against 21 N·cm at control sites. The honest reading is a mixed result — denser and more organised in architecture, less mineral-dense by the quantitative metric, in seven patients — and it is one cohort reported twice, not two series.

The alveolar cleft series grafted deciduous pulp cells on a xenograft scaffold into six patients aged 8 to 12 years, with bone filling of 75.6% (SD 4.8) on CT volumetry at six months [21]. Comparators were historical: eight patients treated with rhBMP-2 and eight with iliac crest graft, drawn from an earlier randomised, observer-blinded surgical trial. The comparator percentages could not be confirmed on repeated retrieval and are not quoted. The finding that matters for interpretation is that the group

difference had disappeared by twelve months.

Pulp and periodontal regeneration in patients

The endodontic application is older and better populated. Revascularisation of necrotic immature teeth by blood-clot induction predates any cell therapy [22] and works, to a degree, without transplanted cells. Meta-analysis of six studies comprising 260 non-vital immature permanent teeth followed 6 to 24 months gives a mean root length gain of 2.43 mm (95% CI 2.05–2.80, $p < 0.00001$) and dentine wall thickening of 0.16 mm (95% CI 0.07–0.25, $p = 0.0007$) over apexification [23]. Against mineral trioxide aggregate apical plugs, pooled survival was 97.8% (95% CI 94.8–100) for regenerative treatment against 97.1% (95% CI 93.7–100), $p = 1.00$, and success 91.3% against 94.6%, $p = 0.58$, from ten randomised trials — with 86% of the regenerative reports and 72% of the apical-plug reports being case reports or case series [24].

Cell transplantation into pulp rests on preclinical work with growth-factor-supplemented pulp cells [25,26] and on inflammation biology that determines whether a pulp repairs or is lost [27]. The human pilot enrolled five patients with irreversible pulpitis, mean age 28.6 ± 10.0 years, each receiving 1×10^6 autologous mobilised pulp cells in atelocollagen with 300 ng of G-CSF. Electric pulp testing was negative in all five before treatment and positive in four of five at both 4 and 24 weeks; CBCT showed functional dentine formation in three of five; pulp volume fell in the two patients reported individually (0.0143 to 0.0125 cm and 0.0110 to 0.0081 cm); no adverse events occurred by 24 weeks [28]. The study was uncontrolled, unrandomised and unblinded, with $N = 5$.

Table 2: Human series using dental pulp cells, by indication, with the control arm and follow-up that determine how much weight each carries; the mandibular cohort is one group of seven patients reported twice, and the necrotic-tooth trial used non-dental cells [2,3,21,31,28,4].

Indication (study)	N and control arm	Follow-up	Key outcome
Mandibular defect [2,3]	7, split-mouth control site, blinded histomorphometry, not randomised	3 y	Compact lamellar bone; high-density fraction 57.7 (15.0)% vs native 69.3 (6.7)%
Alveolar cleft [21]	6, historical controls (8 rhBMP-2, 8 iliac crest), not randomised	12 mo	75.6 (4.8)% bone fill at 6 mo; difference gone at 12 mo
Periodontitis [31]	132 patients, saline control, randomised; 36 double-blind, 96 open-label	180 (14) d	CAL gain 1.67 (1.51) mm vs 1.03 (1.31) mm saline
Irreversible pulpitis [28]	5, no control arm, unblinded	24 wk	Electric pulp test positive 4 of 5; dentine formation 3 of 5
Necrotic mature tooth [4]	36 (18/18), randomised vs root canal treatment; umbilical-cord cells, not dental	12 mo	Cold response 6% to 56%; electric pulp test 17% to 50%

A randomised controlled trial implanted autologous deciduous pulp cells into traumatised immature incisors against apexification, reporting restored sensibility and continued root development in the cell arm [29]; the primary report could not be opened during preparation of this review and no numerical outcome is quoted for it. In periodontics, a randomised controlled trial of pulp-cell micrografts in intrabony defects reported a significant adjunctive benefit at 6 and 12 months [30], again without values that could be verified at source. The largest controlled dataset is allogeneic pulp cell injection in periodontitis: 132 patients and 158 teeth across a 96-patient open-label three-arm randomisation against saline and a 36-patient double-blind saline-controlled phase I, followed 180 ± 14 days. In stage III disease, clinical attachment gain was 1.67 ± 1.508 mm against 1.03 ± 1.310 mm for saline, probing depth reduction 1.81 ± 1.490 mm against 1.08 ± 1.289 mm, and CBCT defect depth change 0.24 ± 0.471 mm against 0.02 ± 0.348 mm, with no serious adverse events [31]. The increment over saline is

approximately 0.6 mm of attachment, which sits below most thresholds for a clinically meaningful periodontal gain.

The field-level tally is the appropriate summary. Twenty-six published human studies of mesenchymal stromal cells for periodontal regeneration exist across ten countries, five of them using dental pulp and two deciduous pulp, and all had fewer than thirty subjects; craniofacial and alveolar bone has three published phase-1 studies with test groups of 5 to 13 patients alongside eleven registered trials; endodontic regeneration has six published studies and one active registered trial [32].

Banking, sourcing and unquantified logistics

The banking argument is where the gap between rhetoric and data is widest. Collection is genuinely opportunistic: of 40 extracted teeth processed for banking, 31 (77.5%) yielded viable cultures, and viable cells were recovered up to 120 hours after extraction, with eight cultures characterised in detail for tri-lineage differentiation and surface markers [33]. That 120-hour window is the practically important number, because it means a tooth extracted in a private surgery can reach a processing facility by ordinary courier.

Beyond that, the commonly quoted logistics figures do not survive checking. Cryopreservation protocols for pulp and for intact pulp tissue have been optimised and published [34], but no post-thaw viability percentage could be confirmed from a primary source, and none is stated here. Nor could any figure for cells recovered per tooth, or for the dependence of yield on donor age, be traced to a primary report; the per-tooth counts circulating in the literature are largely review-to-review citations. A culture-establishment rate is a proxy for yield, not a yield. Any bank quoting recovery or viability numbers to patients should be asked which primary measurement they come from. The qualitative position is defensible —

pulp tissue survives transport, cultures can be established from most teeth, and freezing protocols exist — and it should be stated qualitatively until someone publishes the measurements.

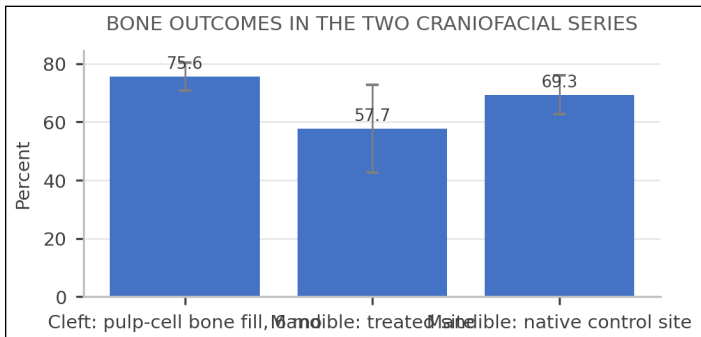


Figure 2: The two human craniofacial bone series: CT bone filling at 6 months after deciduous pulp cell grafting of alveolar clefts (N = 6, historical comparators not plotted) [21], and high-mineral-density bone fraction at treated and native control sites 3 years after split-mouth mandibular grafting (N = 7), where the regenerated bone was less mineral-dense than the patients' own control bone [3].

Conclusion

The laboratory case for dental pulp stem cells is solid within its limits: they are clonogenic at rates an order of magnitude above marrow stromal cells in the founding comparison, they retain odontogenic potential clonally in two-thirds of strains tested, they are recoverable from three-quarters of discarded teeth up to five days after extraction, and they are ontogenetically native to the craniofacial skeleton. The clinical case is not yet made. All craniofacial bone experience is a seven-patient split-mouth cohort reported twice and a six-patient historically controlled cleft series whose advantage vanished by twelve months; the largest randomised dental pulp dataset delivers roughly 0.6 mm of attachment gain over saline at six months; the pulp regeneration pilot has five uncontrolled patients; and the one trial with a genuine double-blind design in regenerative endodontics used umbilical-cord cells. Two conclusions from the primary literature deserve to be carried forward rather than softened: that there is currently insufficient evidence to support clinical translation of dental stem cells into

practice [32], and that 86% of the regenerative endodontic literature consists of case reports and case series [24]. The next useful contribution to this field is a controlled craniofacial bone trial with more patients than the entire published series to date, not another characterisation study.

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