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Neural Crest-Derived Stem Cells From Ovine Palate for Alveolar Regeneration

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Objectives Most dental-related stem cell sources are believed to derive from the neural crest, and because of this there is an increasing interest in the study of their neural crest-derived stem cell (NCSC) characteristics.
The potential of obtaining cell cultures with neural crest resemblance from animal-related tissues has been discussed in the literature. However, most reports include the use of serum-rich conditions and do not describe the potential for osteogenic differentiation, slowing translation to the clinic. Therefore, we aimed to culture and characterize NCSCs from the ovine palate (oNCScs) *in vitro* and *ex vivo* and evaluate their ability to
differentiate into bone cells.
Methods Cultures were established from a varied cohort of sheep samples and grown, as monolayers, in serum-free, and under sphere-aggregation conditions to induce and identify a NCSC phenotype. Ovine NCSC cultures were characterized by immunocytochemistry and reverse transcription quantitative polymerase chain reaction.
Results Monolayer cultures expressed stem cell, neural progenitor, and neural crest-related markers.
Culturing ovine NCSCs as neuro- spheres (ovine NCSCs) resulted in an increased expression of neural crest-related genes. The neural-like phenotype was evidenced by the expression of TUJ1, peripherin, NFH, TAU, SYN1, and GAP43. Our results describe the establishment of ovine NCSC cultures from a large variety of sheep in serum-free medium, as NCSC that differentiate into neural-like cells, and differentiation of ovine NCSCs. NC stem cell sheets had large masses of disorganized calcified material which appeared to be resorbed bone tissue. Lack of osteocytes has been illustrated by Tartrate-resistant acidic phosphatase (TRAP).
Conclusions Therefore, we present here a detailed description of the establishment and characterization of ovine NCSCs grown in serum-free media *in vitro*, and the characterization of their molecular NCSC signature.
In addition, we present an *ex vivo*-proof of concept that these NCSCs, generated under serum-free conditions from palate can derive osteogenic-like cells, proposing them as candidates for bone regeneration treatments.