

Review of a study: Conditioned Medium from Periodontal Ligament Stem Cells Enhances Periodontal Regeneration



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**Summary of the Article**

Mizuki Nagata, Kengo Iwasaki, Keiko Akazawa *et al*. conducted a study of the relationship between periodontal ligament stem cells conditioned medium (PDLSC-CM) and the enhancement of periodontal regeneration. The study took placed at Tokyo Medical and Dental University and was published in the *Tissue Engineering. Part A, Vol. 23, No. 9-10.* in May 2017 (<https://www.liebertpub.com/doi/10.1089/ten.tea.2016.0274>), (<http://doi.org/10.1089/ten.tea.2016.0274>).

Periodontal ligament tissues were obtained from 12 teeth, which were donated from 11 healthy doners. The conditioned medium was concentrated by ultrafiltration from the periodontal ligament stem cells, and three different ratios of concentrated conditioned medium were designated as PDLSC-low, PDLSC-moderate, and PDLSC-high to be compared with control-conditioned medium. According to the experiment, a higher concentrated ratio of PDLSC-CM better enhanced periodontal regeneration than lower ratio and control-conditioned medium. However, the study was not able to identify which protein(s) contributed to the enhancement.

 The study concluded that furthermore studies of PDLSC-CM transplantation need to be developed for potential use in regenerative therapy.

**Article information**

* The title of the article is *“Conditioned Medium from Periodontal Ligament Stem Cells Enhances Periodontal Regeneration”.*
* The authors of this article are Mizuki Nagata, Kengo Iwasaki, Keiko Akazawa *et al*.
* The article was published on the *Tissue Engineering. Part A, Vol. 23, No. 9-10.* (<https://www.liebertpub.com/doi/10.1089/ten.tea.2016.0274>).
* It was published in May 2017, and its abstract can be found in PubMed (<https://pubmed.ncbi.nlm.nih.gov/28027709/>). The article’s DOI is available. (<http://doi.org/10.1089/ten.tea.2016.0274>).
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**Study analysis**

1. **Type of study**
	* The study type of this article is randomized control trials. It was conducted at Tokyo Medical and Dental University, but the date for the experiment is not stated in the article.
2. **Study purpose**
	* According to the authors, the main reason for this study was to observe the regeneration of lost periodontal structures by the transplanted PDLSC-CM. Prior to this study, some information had already been discovered. One such example is that the mesenchymal stem cell’s paracrine effects had been found to support cell growth, and the transplantations of conditioned medium were found to enhance wound healing in the animal model. Also, the mesenchymal stem cells from the periodontal ligament were found to generate tooth-specific attachments. These past discoveries advanced the study by narrowing down the options for the test subject to be used for the experiment. The aim for this study was to identify the potentiality of periodontal regeneration by transplanted PDLSC-CM in the periodontal defect model.
3. **Experimental design**
	* Periodontal ligament tissues were obtained from 12 extracted teeth, premolars or third molars, which were donated from 11 healthy doners. The PDL tissue was minced by surgical knife into 500 microliters, then it was incubated at 37°C for 60 minutes in a water bath with 9.5 mL of digest solution. After the enzyme digestion and the addition of mesenchymal stem cell growth medium, it was passed through a 70-micrometer pore size cell strainer to eliminate debris. The colony of PDLSCs was formed and gathered at 37°C, 5% CO2. The CM finally concentrated from the PDLSCs by ultrafiltration and made three different variables: non (original), once (17-to 31-fold), and twice (450-fold). They were designated respectively as PDLSC-low, PDLSC-moderate, and PDLSC-high, depending on the ratio of CM concentration. Concentrating once with ultrafiltration was measured at around 27-fold concentration. Also, normal human dermal fibroblasts were purchased and concentrated CM once using ultrafiltration. The control group experiment, control-CM, is the serum-free DMEM from the culture dish without cells. Lastly, a 2 mm (height) by 3 mm (width) of surgical periodontal defect was made at the male Sprague-Dawley rats’ buccal area in the mandibular first molar.
	* This study was conducted over time, 4 weeks after transplantations of CM to periodontal defect.
	* The researchers measured the exposed root surface area up to the line connecting the mesial and distal points of the CEJ of the mandibular first molar. They did this by using the 3D reconstructed microcomputed tomography scans and BZ-analyzer software after 4 weeks of each Control-CM, Fibroblast-CM, PDLSC-low, PDLSC-moderate, and PDLSC-high transplantations. They also used a microscope to evaluate the periodontal tissue formation after 4 weeks of transplantation. Lastly, they analyzed the content of PDLSC-CM by three kinds of antibody arrays: angiogenesis-related proteins, growth factors, and cytokines.
	* The researchers analyzed their statistical findings with graphs and pictures.
	* The researchers were calibrated throughout the experiment as the analysis of the experiments was done mostly by software, and standard measurements were the same in each finding.
4. **Results**
	* With respect to the 3D micro-CT, the researchers found out that the PDLSC-CM induces periodontal regeneration. The data further showed that the alveolar bone around the distal root of the first mandibular molar grew more with PDLSC-moderate and PDLSC-high than with PDLSC-low, control-CM and fibroblast-CM. Through use of the microscope, the researchers found more new periodontal tissue formation with PDLSC-moderate and PDLSC-high. As for the contents in PDLSC-CM, angiogenesis-related factors, such as TIMP 1, VEGF, and Pentraxin 3 were found. Growth factors such as IGFBP 6, IGFBP 2, and PDGFR β were found. Cytokine such as Serpin E1, MCP-1, and MIF were found. The decreased ratio of Tumor Necrosis Factor- α and inflammation-related molecules, IL-6, IL-1β, and COX-2 shows that PDLSC-CM exhibits anti-inflammatory effects during periodontal wound healing.
	* The results were not statistically significant because the main purpose of this experiment was to identify whether PDLSC-CM induces periodontal regeneration or not. Also, the authors did not discuss any possibilities or percentages, but they displayed bar graphs only to compare with other variables.
5. **Conclusion**
	* The authors concluded that transplantation of PDLSC-CM into rats’ periodontal defect induced periodontal regeneration, and regeneration was enhanced when the concentration ratio of CM was moderate and high. Additionally, they stated that even though they were unable to identify all the contents of PDLSC-CM, the result from antibody arrays demonstrated that the PDLSC-CM contains many different proteins, and the level of mRNA of TNF- α in periodontal tissues decreased significantly.
	* Authors pointed to the need for further investigations, which should focus on identifying the specific contributing factors that allow PDLSC-CM to induce periodontal regeneration. Also, a different experimental time frame is needed in order to be certain about the regeneration mechanism. Limitations for further study are not mentioned in the article.
6. **Your impression**
	* I think this study is important and should be a starting point for further investigation because as of now, periodontitis is irreversible. If we find a way for stem cells to be transplanted without any risk factors and regenerate the periodontal bone, then periodontitis can be cured. This can be one of the treatments for patients who suffer from periodontal bone loss. Also, when the specific protein(s) or components of PDLSC-CM that directly contribute to periodontal regeneration are successfully identified, I believe other related studies, such as studies into bone ossification, will accelerate. Therefore, if I had the opportunity to research and learn more about this topic, I would focus on identifying the components of PDLSC-CM and the factors that initiated the process of regeneration at the periodontal defect area. I hope further research based on this study will improve the quality of life of all those suffering from bone loss, regardless of their financial resources to seek treatment.