Comparative Histopathologic Analysis of Granulomatous Tissue of Endodontic and Periodontal Origin

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Purpose: The aim of this study was to compare the percentage of tissue types and assess the presence/absence of odontoblasts or preodontoblasts in granulation tissue harvested from lesions associated with teeth extracted due to endodontic and periodontal reasons. Materials and Methods: Histologic reports of cases with a confirmed diagnosis (ie, endodontic or periodontal diseases) were included. These should include a semiquantitative analysis of the percentage of tissue types per sample (ie, epithelium, subepithelial connective tissue, bone or chronic inflammation/deep connective tissue). The overall percentage of tissue type per diagnosis was calculated. Quantitative variables were summarized with means and standard deviations. Normal distribution was tested by the D’Agostino-Pearson omnibus normality test. The level of \( P < .05 \) was adopted for statistical significance. Finally, an analysis of the salient findings was summarized. Results: The reports from 19 patients were included, 9 of endodontic and 10 of periodontal origins. The granulomatous tissue of endodontic and periodontal disease origin was similar, and consisted mainly of chronic inflammation (endodontic 40%, periodontal 41.7%), followed by epithelium (endodontic 25.7%, periodontal 20.8%), subepithelial connective tissue (endodontic 18.6%, periodontal 20.8%), and bone (endodontic 15.7%, periodontal 8.3%). No significant differences were found when comparing the groups regarding the percentage of tissue types \( (P \geq .05) \). No osteoblasts or preosteoblasts were reported. Conclusion: Within the limitations of the study, the granulomatous tissues associated with chronic infection of endodontic or periodontal origin are comparable and consist primarily of chronic inflammatory cells. Int J Oral Maxillofac Implants 2020;35:585–590. doi: 10.11607/jomi.8076

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In the presence of infective processes such as periodontal, endodontic, or endo-periodontal lesions, healing following tooth extraction may be hindered, with greater alveolar process volume reduction compared with sites uncompromised by chronic infection. Following extraction in an uncompromised site, the socket is filled with a blood clot,\(^1,2\) which is then almost entirely remodeled and substituted with granulation tissue within the first week. Over the next 2 weeks, there is progressive replacement of the granulation tissue by connective tissue.\(^2,3\) Conversely, in the case of chronic infection and a subsequent inflammatory immune response, the alveolar socket walls are covered by granulation tissues, with the blood clot containing inflammatory cells that are remnants of the response to the original infective process.\(^4–8\)

The histopathology of compromised sites has been previously studied. Analysis of granulation tissue around periodontal lesions demonstrates the presence of high concentrations of neutrophils and some macrophages during the acute phase, with lymphocytes, macrophages, and plasma cells detected in the chronic phase of the disease process.\(^4\) There is no homogenous or site-specific pattern of distribution of infiltrating cells. Histologic analysis of apical periodontitis exhibits a dense infiltration of immunocompetent cells with the majority of inflammatory infiltrate consisting of T and B lymphocytes and macrophages.\(^5,6\) During the acute phase, lesions are infiltrated mostly by polymorphonuclear leukocytes, whereas in the chronic phase, there are predominantly plasmocytes, lymphocytes, macrophages, and mast cells.\(^7\) Finally, in endodontic-periodontal lesions, there is involvement of both the periodontal, pulpal, and/or periapical tissues with the presence of reactive soft tissues that have histopathologic features similar to those present in periapical and periodontal infected tissues.\(^8\)

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The use of grafting materials has been proposed to prevent bone resorption after tooth extraction. Autogenous bone is commonly described as the “gold standard” because of its osteoconductive, osteoinductive, and osteogenetic properties, though an appropriate donor site is required and the procedure is associated with postoperative morbidity. Therefore, bone substitute grafts and/or membranes are often preferred clinically.9–11

Factors normally proposed as important for ensuring successful socket preservation include asepsis, the complete removal of reactive granulation tissue, an adequate blood supply, and primary soft tissue closure.12 Conversely, some authors have proposed maintenance of the reactive soft tissue in the alveolar socket (ie, granulation tissue) for stimulating bone regeneration without the placement of a bone substitute graft or biomaterial.13,14 The rationale behind this concept is that if the granulation tissue formed postextraction differentiates into bone, then some precursor cells should be present.15,16 Multipotent progenitor stem cells have been detected in infected granulation tissues from chronic periodontitis lesions,17 and it has been purported that these have a similar capacity to stimulate bone formation, as those present in the granulation tissue formed after tooth extraction.13 However, it is worth noting that what can be described clinically as granulation tissue may differ histologically, and it will be of clinical significance to assess these, aiming to evaluate the osteogenic regenerative potential of tissues found in compromised sites due to chronic infection.

Therefore, the aim of this study was to compare the tissue types (ie, epithelium, subepithelial connective tissue, bone or chronic inflammation [including deep connective tissue]) and assessment of the presence/absence of odontoblasts or preodontoblasts in granulation tissue harvested from lesions associated with teeth extracted due to endodontic and periodontal reasons. The null hypothesis was that there are no differences in tissue types in granulation tissue of endodontic or periodontal origins.

**MATERIALS AND METHODS**

The study was based in a private multispecialty dental practice in Rome, Italy, in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (https://www.wma.net/wp-content/uploads/2018/07/DoH-Oct2008.pdf) and the additional requirements of Italian law.

Previously anonymized histologic reports prepared by an experienced pathologist (A.S.) ordered from a dental practice were retrospectively examined in the present study. The records were anonymized as they were no longer required. As such, the study was exempted from ethical review by the University of Adelaide (Australia), as it carried only negligible risk and involved the use of existing data that contains nonidentifiable data.

To be included, the reports should have histologically assessed the granulation tissues associated with teeth extracted because of a clinical diagnosis of periodontal or endodontic disease made by one of the authors (L.C.). Reports related to samples with an unclear or different diagnosis, or if a diagnosis was not stated in the clinical notes section of the related request form, were excluded. Based on the diagnosis present in the request form, for the present study, the included reports were allocated to either the “periodontal” or “endodontic” group. Originally, the relevant lesions were diagnosed as of periodontal origin if the tooth in question presented with pocket probing depths ≥ 3 and/or furcation involvement, positive response to cold pulp sensitivity testing (Endo Frost, Roeko), and when radiographic examination suggested vertical and/or horizontal bone loss. Conversely, lesions related to teeth without pocket probing depths ≥ 3 and/or furcation involvement, negative response to pulp sensitivity testing, and when radiographic examination showed an apical radiolucency associated with the tooth in question, were considered pathoses of endodontic origin. All teeth were extracted in preparation for implant placement following discussion with the patients regarding diagnosis and possible treatment options, as part of the shared decision-making process. To harvest the samples, the extraction sockets were curetted to recover any retained granulation tissue using Miller Surgical curettes (Hu-Friedy Italy). Finally, reports were included if they contained a scoring system for types of tissue present in the samples, which is described below.

**Histopathology**

Originally, the tissue samples were fixed in 10% neutral buffered formalin for 24 to 72 hours, then dehydrated in a graded series of ethanol rinses, and finally embedded in paraffin. Serial sections were subsequently stained with hematoxylin and eosin and observed under a light microscope.

The following types of tissue were assessed:

- Epithelium (presence, hyperplasia, degenerative changes, exocytosis)
- Subepithelial connective tissue (most superficial part of the lamina propria, in close contact with the basement membrane on which the epithelium rests [presence, degenerative changes, inflammation, neoangiogenesis])
- Bone (presence, degenerative changes)
• Chronic inflammation (macrophages, lymphocytes, and plasma cells), associated with other events (neoangiogenesis, fibroblastic proliferation and maturation, deposit and remodeling of the extracellular matrix) and deep connective tissue (presence, degenerative changes, inflammation, neoangiogenesis)

**Semiquantitative Analysis**

Each sample was scored from 0 to 4 for the aforementioned types of tissue as follows: 0 = absence; 1 = presence up to 25% of the entire surface; 2 = presence between 25% and 50% of the entire surface; 3 = presence between 50% and 75% of the entire surface; 4 = presence between 75% and 100% of the entire surface. Subsequently, the percentage of the tissue type per group (ie, endodontic or periodontal) was calculated by adding the scores of each sample per tissue type and dividing this value with the scores from all types of tissues related to the same group.

**Statistical Analysis**

Quantitative variables were summarized with means and standard deviations. Normal distribution was tested by the D’Agostino-Pearson omnibus normality test. One-way analysis of variance (ANOVA) was used for comparisons between groups. Statistical analyses were performed using the SPSS 21.0 statistical package (SPSS). The level of \( P < .05 \) was adopted for statistical significance.

**RESULTS**

Nineteen histologic reports fulfilling the inclusion criteria were identified. Nine were “endodontic,” and the remaining 10 were “periodontal.”

**Percentage of the Tissue Type Per Group**

The most common tissue type was inflammation in both groups. In the endodontic group, these were: inflammatory cells, 40.0%; epithelium, 25.7%; subepithelial connective, 18.6%; and bone, 15.7%. In the periodontal group, these were: inflammatory cells, 41.7%; epithelium, 29.2%; subepithelial connective, 20.8%; and bone, 8.3%. No significant differences were found when comparing the groups regarding the percentage of tissue types (\( P \geq .05 \)). Percentages of tissue types are summarized in Fig 1.

**Narrative Synthesis of Salient Findings**

**Endodontic Group.** Bacteria and acute inflammatory infiltrate were not observed, but a chronic inflammatory infiltrate was present in all specimens. Leukocytes and plasma cells were present in the specimens, and low levels of neutrophils and monocytes were found in all specimens. Mild edema of the stratum spongiosum with the vacuolization of the epithelial cells was evident along with hyperemia of the underlining lamina propria. Discrete presence of giant cells was observed in two specimens. Bone and osteoclasts were observed in a few areas, in the absence of osteogenesis or preosteoblasts. Endothelial sprouting and early stage angiogenesis were observed. The epithelial lining was continuous and well developed, when present. Some fragments of necrotic bone, surrounded by a fibrotic reaction, were seen. Figures 2 to 4 are representative of the described histologic findings in different endodontic samples.

**Periodontal Group.** The epithelial layer appeared hyperplastic and edematous. The underlying lamina propria held a dense inflammatory infiltrate, mainly consisting of mononuclear cells. A large bacterial aggregate was observed, surrounded by polymorphonuclear cells. Hyperplasia and edema of the oral epithelium were observed. However, mild and severe lymphocyte-histiocyte-plasmocyte infiltrate was found in some areas. The tissues contained a larger infiltrate of leukocytes and plasma cells. In many areas, low levels of neutrophils were observed, and low levels of monocytes were found. Giant cells were absent in two specimens, though their moderate and discrete presence was observed in some specimens. No preosteoblasts or osteoblasts were detected in any histologic field, though some osteoclasts were found. These results suggest that there was chronic inflammation in the tissues assessed. Figures 5 to 7 are representative of the described histologic findings in different periodontal samples.

![Fig 1](image)
DISCUSSION

The overall findings suggest that granulomatous tissue from endodontic and periodontal disease is similar and consists mainly of chronic inflammatory cells, attributable to a high leukocyte content. Therefore, the null hypothesis could not be rejected. This is of clinical relevance, as chronic inflammatory diseases of almost any cause are associated with bone loss and the absence of osteogenic regenerative potential.18 Similarly, according to the reports, preosteoblasts or osteoblasts were not observed, but some osteoclasts were detected. Overall, the reported findings are associated with bone resorption and are sufficient to exclude the osteogenic potential of the harvested tissues.

The role of maintaining postextractive granulation tissue with regard to bone healing remains controversial. It has been suggested that granulation tissue related to apical periodontitis contains osteogenic cells that have the potential to differentiate and produce calcified deposits in vitro.19 Maeda et al proposed that such granulation tissue does not include mature osteoblasts and/or cementoblasts, but preosteoblasts or precementoblasts, which subsequently differentiate into mature cells that increase the expression of osteogenic markers.19 Similarly, Hung et al isolated stem cells from human periodontal granulation tissues and reported that these improved bone formation when they were inserted into mice calvaria defects.20 Nevertheless, further studies are needed to demonstrate similar efficacy in humans. Furthermore, Ronay et al argued that osteogenic cells are present in the reactive soft tissue because granulation tissue is formed in the alveolus following tooth extraction and subsequently changes into bone.17 They found that cell cultures from gingival granulation tissue expressed embryonic stem cell markers,17 though it was not clear how this marker contributes to differentiation and bone formation in vivo. Conversely,
Trombelli et al histologically assessed alveolar healing in humans and found the formation of granulation tissue; however, they suggested that it cannot be assumed that granulation tissue present in the alveoli at the time of extraction has regenerative potential.

After chronic inflammatory tissue, the largest percentage of tissue found was epithelium and connective tissue, which can be beneficial by protecting the alveolus when the socket is filled with graft material aiming for alveolar preservation. Mardinger et al aimed to use the reactive soft tissue present inside the alveolus, keeping it attached to the vascular pedicle, to obtain and maintain primary closure of the alveolus after the use of a bone replacement material. In this way, the creation of a covering tissue was promoted, with the histologic analysis showing the characteristics of keratinized gingiva, due to the rapid proliferation of the long junctional epithelium present in the original periodontal pocket.

It should be noted that in the present study, a relatively small percentage of bone was reported; however, this is possibly derived from bone scraped during sampling via curettage. This is because in the absence of osteoblasts or their precursors, osteogenic activity is not expected. Osteoblasts are responsible for new bone formation with a spherical, large nucleus, and the cells are arranged in a row. They are identified through hematoxylin and eosin staining, which shows that the cytoplasm of active osteoblasts is slightly basophilic due to the substantial presence of rough endoplasmic reticulum. Preosteoblasts are precursor cells that are committed to the osteoblast lineage. It should be noted that general chronic inflammatory processes influence osteoclasts and osteoblasts through the interaction of hormones, cytokines, and growth factors with bone cells.

A difference noted was that in lesions of periodontal origin, bacteria associated with acute inflammatory infiltrate were reported; however, this was not the case for pathoses of endodontic origin. This is consistent with previous literature, as chronic apical periodontitis is usually associated with the presence of root canal infection, rather than extraradicular infection, as the latter is considered uncommon, whereas periodontal lesions are close to surfaces colonized by biofilms.

The main limitations of the present study are represented by the limited sample size and by the study design, being retrospective, with the latter being a possible source of bias. Furthermore, no molecular biology investigations were carried out to explore the presence of embryonic stem cells, and no specific markers were used. However, it should be noted that the described types of tissues were consistent throughout the included histologic reports, and the methodology is used routinely in pathology services in the clinical reality. In fact, the present study used reports originating from the secondary care setting.

The main clinical implication of the present study is that leaving granulation tissue in the alveolus, which is composed mainly of chronic inflammatory tissue and secondarily of epithelium-connective tissue, is unlikely to bring any advantage in an osteogenic sense, but may have a potential role as an epithelial-connective tissue sample to hasten primary closure, because of the type of tissues found. Previous clinical studies support leaving reactive soft tissue in situ, which, in association with grafted collagen sponge, may prevent the collapse of the fresh socket after tooth extraction. However, these studies present several limitations, such as the absence of a control group and a limited recall period. Therefore, the authors propose that if granulation tissue of endodontic and/or periodontal origin remains in the socket, the inflammatory process will require time for resorption, and thus, will delay the process of alveolar healing. This has been previously demonstrated in endodontics. Also, considering the potential risk of timely consecutive retrograde peri-implantitis of endodontic origin, adequate local measures are recommended before implant placement, even if the prevalence of this complication appears to be low. Possible causes of retrograde peri-implantitis of extraradicular origin include granulomatous tissues, cysts, scar tissue, and extraradicular infections; however, the literature assessing this subject is scarce, and diagnosis of these lesions is demanding.

CONCLUSIONS

The granulation tissue present in extraction sockets related to the presence of periodontal and endodontic lesions is comparable and consists of chronic inflammatory cells. No osteoblasts or preosteoblasts were present.

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REFERENCES


