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Review Article

The Applications of Stereology in Oral and Maxillofacial Medicine

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Abstract: *Purpose:* To provide information for the further application of the quantitative research in oral and maxillofacial medicine. *Methods:* Reviewed the literatures related to the applications of quantitative stereology in the subdisciplines of the oral and maxillofacial medicine and other medical specialty both in China and in other countries since 1970s. Results: Stereological method as a kind of unbiased mathematical method, can accurately calculate the volume of a specific object, region, length, surface area, etc. As an interdisciplinary science, stereology is closely linked with very more other disciplines. While compared with other medical specialty, there are much deficiencies, such as narrow range of application, less stereological parameter relative, to be improved in the applications of quantitative stereological technique in oral and maxillofacial medicine. *Conclusion:* Currently stereological theory and method have not yet been well applied in oral and maxillofacial medical research. In the future research, it should expand quantitative stereological method in the application of oral and maxillofacial medical, and exert the advantages of stereology and further explore new stereological parameters, pay attention to the joint application of a variety of parameters.

Keywords: Stereology, Oral and Maxillofacial Medicine, Oral Pathology, Oral Anatomy

1. Introduction

The word "stereology" earliestly created by a few biologists and mathematicians in Germany in 1961, is intended to guide people to explore the three-dimensional structure of the cross section [1]. Stereological research articles began to appear in the world until 1965, while China began to emerge research articles about high levels of stereology until 1985 [2]. In recent decades, with the continuous improvement of modern stereological methods, and the constantly expanding of the applications of stereology, the frequency of "stereology" appears in the literature are also increasing [3].

Compared against the other medical specialty, the number of applications of the stereology in Oral and Maxillofacial medicine is comparatively small, and the applied range is relatively narrow. The purpose of this article is to review the extensiveness, advantages and reliability of the applications of the quantitative stereology in oral and maxillofacial medical, which would provide information for the further application of the morphologic quantitative technique, such as stereology, in oral and maxillofacial medicine.

2. Stereology Characteristics

Stereology is a new and highly cross but independent discipline, having recognized subject name and obvious characteristics both in China and abroad. According to different disciplines, the definitions of stereology have a variety of effective kinds, while the core content of the basic definition is "research on three-dimensional (stereo) or three-dimensional (stereo -) structure ". Biomedical stereology is an emerging interdisciplinary science, which obtains the three-dimensional quantitative information of the

structures by analyzing the two-dimensional data of the tissues, organs and/or cells. It provides information on 3-dimensional structures, such as volume, volume density, surface density, number density, etc, by testing quantitatively the geometry information of the plane image (such as particle cross section area, perimeter, diameter, density, node density, etc.), and in accordance with the relevant stereological formulas, so that people can understand the structure characteristics of tissues and cells from the level of the 3-dimensional [4].

Stereology is an effective and objective method that avoids systematic bias. Mathematical unbiasedness is an inherent property of the design of stereological methods that does not need to be, and cannot be, proven by experiment [5]. By using stereological methods, volume, surface area and curve length are estimated free from any systematic error (i.e. bias) relating to the sampling strategy and without the need for any assumptions regarding the shape of the structure being investigated [6]. Stereology method is fast, high accuracy and good repeatability in measuring the parameters of the tissues and organs. If the measurement process is repeated, the average of all trials will be the true value of the quantity of interest [3, 5].

The basic tools of stereology test (measurement) are points, lines, boxes (surface) of three geometrical elements, or "probe" (otherwise known as the test system). Stereology test is essentially equivalent to put the "probe" randomly "insert" the structure of the organ tissues, and then according to the result of detection (measuring point is located within the measured structure, measuring line crosses over the measured structure and other basic test results) to analyse statistically the geometrical characteristics of the tested structure. Stereological basic test method includes measuring point counting, intersection counting, contour counting, particle counting, the Feret diameter measurement, focal length measurement and take intercept point measurement, etc [4, 7].

Stereology includes four basic parameters: (1) density parameters: quantitatively describing the shape and the number of units of a reference in structural components, including the volume density, surface density, curvature density, length density, number density, etc; (2) shape parameters: a quantitative expression of the shape of the tissue structure, including: the particle surface area volume ratio, axial ratio, the average surface area, mean curvature, sphericity, etc; (3) dimension parameters: quantitatively reflect the size of the structural components of the tissue, including: the average particle diameter, average volume, average intercept, the barrier thickness; (4) the distribution parameters: quantitatively measure the spatial structure, including: the mean free path of the particles, particle dispersity, the average center distance, the nearest neighbor distance. In addition to these four basic parameters, there also have the total number of particles, the total surface area of the structural component, the total volume etc. For application of these parameters, morphological problems related to almost all biomedical disciplines can be quantitatively studied, but must using the appropriate parameters according to the structural characteristics of the organization to be quantified [7].

3. The Applications of Stereology

3.1. The Applications of Stereology in Oral and Maxillofacial Medicine

Stereology, as an interdiscipline, has very close ties with many disciplines, including anatomy, embryology, pathology, immunology, pathophysiology, pharmacology and toxicology, molecular biology, genetics, imaging, and epidemiology [8-12]. The applied fields of the stereology in abroad is relatively wider than it is in China, including oral tumor pathology, oral implantology, periodontology, disease of the oral mucosa and oral materials science, oral anatomy and other related disciplines [13-17]. While the applications of stereology in Chinese oral and maxillofacial medicine are more concentrated in basic clinical medicine, mainly including oral pathology and oral anatomy. In addition, in medical radiographic imaging, imaging technology such as CT (computed tomograph), MRI (magnetic resonance imaging) and ultrasonography can obtain a series of images, which all can meet the unbiased stereology statistical sampling strategy. Researches also prove that the combination of the quantitative stereological inspection technology based on the Cavalieri principle with CT, MRI and ultrasound technology will make the examinations be fast, objective, noninvasive, and high accuracy [18-20]. The applications of stereology in oral radiographic studies in recent years are also provided relevant reports [21], but a relatively small number. Compared with the other medical specialty, the number of applications of the stereology in Oral and Maxillofacial medicine is relatively small, and the application range is relatively narrow. However, scholars in recent years are gradually paying more attention to the sterehiological applications.

3.2. The Applications of Stereology in Oral Pathology

Stereological technology has been used in some tumor cells with morphological quantitative research, that lay a foundation for the quantitative analysis of the pathological diagnosis and judging prognosis. Besides, stereology has great practical value on the cancer cells gradual change process, extent of tissue damage and analysis of the prognosis of patients, etc [22-24]. Stereology can obtain tissue section microstructure of 3d data (including volume, surface area, length, and the number of tissues, organs, cells and organelles, etc.), and these quantitative information are useful for evaluating the experimental treatment effect of the special organs, tissues and cells [25].

Stereological method can be used to examine the clinical classification of oral verrucous carcinoma (OVC) and to see for any difference in the biological behavior between OVC and Squamous cell carcinoma (SCC). Stereology could be used to measure and describe the morphological parameters of the nucleus to cytoplasm ratio (Vnp), desmosomes and mitochondria. The nucleus volume density (Vv), Vnp,

desmosomes and intracellular desmosomes number density (Nv), were also observed by stereology [26]. Stereological technology also could be used to analysed the evolutionary changes, from normal oral mucosa tissue to epithelial dysplasia of oral lichen planus and oral leukoplakia, and enventually to oral well-differentiated squamous carcinoma tissues [27]. Results showed that the two-dimensional parameters could be better reflecting the characteristics of the cell morphological changes in the process of malignant transformation, while the three-dimensional parameters were highlighted the nucleus in tumor characteristics in the process of changes. The combination of two-dimensional and three-dimensional quantitative parameters could more fully reflect the cell and the nucleus of oral mucosa epithelium from normal to lesion to cancer quantitatively, and could be well to distinguish oral cancer from the benign lesion.

Stereological techniques also used in the quantitative morphological study of gum and salivary gland tissue with injury or disease [16, 28, 29]. London B et al [28] examined 13 gingival biopsies taken from 13 renal transplant patients (mean age 26.5 yr), 11 of whom exhibited cyclopropane A (CsA) -induced gingival overgrowth by stereological methods. The study showed that the volume density of oral epithelium and the surface density of epithelial ridges in the CsA-induced gingival overgrowth were significantly increased compared to normal gingival tissue. Caldeira et al 16 used the stereological techniques to test the Ultrastructures of the salivary glands of Diabetic Nod Mice submitted to long-term insulin treatment, included nuclear and cytoplasmic volumes, long and short axes, and so on. Li Jian et al [29] studied the structural changes of the traumatic parotid gland of the rat after being treated by bFGF using the quantitative stereological calculation methods. The results showed that the absolute values of the acinar area, the quantity of the mesenchyme and duct, the nucleoplasm ratio and other indexes in the treatment group were better than the control group. The study explored innovative ideas of the prevention and treatment of parotid fistula.

Stereological techniques often combined with other techniques to obtained the quantitative characteristics of the object [30, 31]. Li Jun et al [30] combined the stereology with radiology, histology and other techniques to investigate the effects of dexamethasone on repair of a critical size defect of the mandible in male Sprague-Dawley rats. They found that short-term systemic administration of dexamethasone impaired the early bone healing process in a critical sized mandible defect model. Schou S et al [31] used stereological method and other techniques to evaluate the effect of anorganic porous bovine- derived bone mineral (Bio-Oss) and expanded polytetrafluoroethylene (ePTFE) membrane in the treatment of peri-implantitis. They found that the amount of re- osseointegration and the total amount of bone (Bio-Oss and regenerated bone) were significantly higher in defects treated with membrane-covered Bio-Oss as compared with the Bio-Oss, membrane, or a conventional flap procedure (control) only.

Clinically, there are different viewpoints about the quantitative judgment of tumor or other measuring targets between different doctors. Using stereological method to estimate can avoid the influence of subjective and psychological factors, as well as improve the efficiency and accuracy of the targeted three-dimensional information.

3.3. The Applications of Stereology in Oral Anatomy, Science of Dental Materials and Oral Microorganism

Stereological method as a kind of unbiased mathematical method, can accurately calculate the volume of a specific object, region, length, surface area, etc [32, 33]. Stereology, as an interdiscipline, has very close ties with many disciplines, including anatomy, embryology, pathology, immunology, pathophysiology, pharmacology and toxicology, molecular biology, genetics, imaging, and epidemiology [8-12], especially with neuromedicine [34-36].

Barghash Z et al [37] used the stereological methods to evaluate the degeneration and regeneration of the facial and mental nerves after a crush injury at the histological level. They found differences between the two nerves both in the normal anatomy and the regenerative pattern. White WM et al [38] designed a pilot observational cohort study comparing noninvasive imagings of human oral mucosa obtained by confocal reflectance microscopy in vivo with histology by using stereological methods. The result was confocal images correlated well with conventional histology, both qualitatively (visual analysis) and quantitatively (stereology). Tan Weibing et al [39] analysed the three-dimensional structure of the imperceptible blood-vessel located in the superficial dermal layer, the deep dermal layer and the superficial fascia layer under the deep dermis of the muffle and the lower lip area by using the quantitative stereological methods. They compared respectively the imperceptible blood-vessel length of the density (Lv), the imperceptible blood-vessel diameter (D), and the imperceptible blood-vessel surface area density (Sv) of the two area. The stereological study of the temporalis muscle spindle found that the distribution of the temporalis muscle spindle was heterogeneous and tufted, especially the front temporal muscle fiber near the coracoid [40, 41]. Mackert JR Jr et al [42] provided evidences that microcracking in dental porcelain can be minimized by a reduction of the mean leucite particle diameter to less than 4 microns by analyzing the microcrack density, leucite particle surface area per unit volume, and leucite mean volume-surface diameter, D3,2, using quantitative stereology. Dige I et al [43] designed a study to develop and evaluate a stereological method for quantification of bacteria in intact biofilm, which was the first time a mathematical design-based stereological approach had been used to quantify bacteria in situ grown dental biofilms. The study demonstrated that the combined use of FISH and stereology was a relevant and reliable tool for obtaining unbiased information about the numerical contributions of specific bacterial populations during initial biofilm formation [43].

According to the results of literature retrieval, stereology technology in different areas of the oral medicine abroad has

very extensive applications, such as oral tumor pathology, oral implantology, periodontology, disease of the oral mucosa and oral materials science, oral anatomy and other related disciplines [13-17], while the applications of stereology in Chinese oral medicine mainly focus on oral pathology, followed by oral anatomy. Anyway, Compared with the other medical specialty, the applications of the stereology in Oral and Maxillofacial medicine still have limitations.

4. Discussion

To sum up, stereology, based on the mathematical principle of geometry, is a type of quantitative measurement, which is fast, unbiased, repeatable and non-invasive [5, 32, 33]. Stereology image analysis technology can test results and quantify the relative changes in the tissue structure, which can be widely used in various fields of oral and maxillofacial medicine. Quantitative stereological method is helpful to the diagnosis of benign and malignant lesions, the treatment and prognosis of malignant tumors. It can provide quantitative parameters and standard for the differential diagnosis of the pathological changes of tumor and tumor-like lesions, benign tumors and malignant tumors [26, 27, 44, 45]. Stereology technology can also be combined with oral radiological imaging, such as CT, MRI and ultrasound technology, using for quantitatively describing the normal anatomy of oral and maxillofacial region and the imaging characteristics of benign and malignant lesions, and also using for quantitatively describing tissue defect after tumor surgery and trauma range and damage degree, etc, what will provide reliable data for the tissue reconstruction after oral and maxillofacial surgery and trauma. In recent years, whether the application of modern body quantitative stereological method, has become a necessary criterion of biomedical research papers of Science, Neurobiology of Aging, the Journal of Neuroscience and other internationally famous magazines.

5. Conclusion

Currently stereological theory and method have not yet been well applied in oral and maxillofacial medical research. Compared with other medical specialty, there are several shortcomings, such as narrow range of application, less stereological parameter relative. In the future research, it should expand quantitative stereological method in the application of oral and maxillofacial medical, and exert the advantages of stereology and further explore new stereological parameters, pay attention to the joint application of a variety of parameters.

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