

## Impact of Histochemistry on biomedical research: looking through the articles published in a long-established histochemical journal

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### Abstract

Histochemistry provides the unique opportunity to detect single molecules in the very place where they exert their structural roles or functional activities: this makes it possible to correlate structural organization and function, and may be fruitfully exploited in countless biomedical research topics. Aiming to estimate the impact of histochemical articles in the biomedical field, the last few years citations of articles published in a long-established histochemical journal have been considered. This brief survey suggests that histochemical journals, especially the ones open to a large spectrum of research subjects, do represent an irreplaceable source of information not only for cell biologists, microscopists or anatomists, but also for biochemists, molecular biologists and biotechnologists.

### Introduction

During the last five years, more than 60,000 articles have been published where *Histochemistry* is present as MeSH (Medical Subject Headings) Term (see, <http://www.ncbi.nlm.nih.gov/>). This apparently demonstrates that histochemistry represents a key tool in biomedical research, for a wide variety of topics.

Obviously, the strictly histochemical Journals are the richest source of information for all those scientists who regularly use microscopy and histochemistry in their research; it is likely that these investigators included histochemical articles in the reference lists of the papers they have published.

In the attempt to estimate the impact of histochemical articles in the biomedical field, the last few years citations of articles published in the *European Journal of Histochemistry* have been considered. This sample may be taken as a reliable -though limited- reference, as the *European Journal of Histochemistry*, since its foundation in 1954 as the official organ of the

Italian Society of Histochemistry, accepts articles by scientists working in a wide variety of biological and medical fields, from cell and tissue biology, to microscopy, cytometry, zoology, human and veterinary medicine, ecology, botany.<sup>1</sup>

### How did the article subjects change in the *European Journal of Histochemistry* during the last 60 years

At the risk of oversimplifying, the published articles have been arbitrarily divided into ten categories, based on the most frequently found subjects: i) Methods; ii) Neurobiology; iii) Connective tissue, bone & cartilage; iv) Muscle tissue; v) Human pathology; vi) Animal biology & Experimental medicine; vii) Development & Stem cells; viii) Cell proliferation and death; ix) Human & Comparative histology; x) Regenerative medicine. In Figure 1, the percentage of the different categories were reported considering the first ten years of publication of the Journal (1954 to 1963), the period 2000-2009, and the last 5 years (2010-2014). In the first ten years of the journal, when histochemistry was living its pioneering age, there was a need to establish staining protocols and define the most appropriate experimental conditions for the application of histochemical techniques to different tissues from a variety of organisms: as a consequence of this intense effort, about 25% of the papers dealt with methods and techniques in the period 1954 to 1963. The articles on *Methods* still represents about 13% of the published ones since 2000 to present, and this demonstrates that a continuous adjustment and refinement of the analytical methods is necessary, to allow investigating heterogeneous cell and tissue models under different experimental conditions.<sup>2-13</sup> Fluorescence microscopy and spectrofluorometry were used for localizing different chemical species,<sup>14,15</sup> and for assessing changes in microenvironmental condition;<sup>14,16</sup> it is worth noting the increasing attention for ultrastructural cytochemistry, which was used (often by multiple-labelling procedures) to detect specific molecules at high resolution.<sup>17-21</sup>

The most popular subjects (more than 40%, as a whole) have always been *Human pathology* and *Animal biology & Experimental medicine*; however, histochemistry was applied to very different targets, gradually over time. Descriptive articles on normal and pathological tissues in humans or animal species were very frequent in the first years, when the relatively poor knowledge of the molecular compo-

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sition of healthy and diseased organs made the detection *in situ* of different chemical components a very powerful diagnostic tool. Recently, most of the published articles aimed to identify diagnostic indicators<sup>22-33</sup> or the progression markers<sup>34-37</sup> of diseases, or at elucidating the pathogenetic molecular mechanisms;<sup>38-45</sup> as a methodological approach, (often multiple) immunohistochemical techniques were used.<sup>46</sup> On the contrary, the percentage of articles on the application of histochemistry to *Neurobiology*, *Development & stem cells*, and *Human & Comparative histology* did not change significantly in comparison with the long-past years. Immunohistochemical techniques were recently used to describe the topographical distribution of nerve cell populations in vertebrate and invertebrate species,<sup>47-51</sup> and to investigate the effects of aging or experimentally-induced stress on the central nervous system.<sup>52-56</sup> The maturation and elimination of oocytes was studied in mammals,<sup>57-59</sup> while specific proteins were investigated during the pre- and neo-natal development,<sup>60-72</sup> in particular of kidney<sup>60,61</sup> and heart.<sup>62,63</sup> Descriptive articles have also been published on the expression of different molecules in Vertebrates organs.<sup>73-81</sup>

In recent times, the papers on *Connective tissue*, *bone & cartilage* became numerous: this indicates that the histochemical approach is presently essential for studying structure and function of the hard tissues.<sup>82,83</sup> The unique structural characteristics of bone and cartilage makes it often necessary to use, in an integrated approach, immunocytochemistry, transmission and electron microscopy, as well as physical and biomolecular techniques.<sup>84-88</sup> In several papers, qualitative and quantitative assays were used to study the dynamics of tooth repair both *in vivo* and *in vitro*, with special reference to the secretion and accumula-

tion of dentin matrix proteins.<sup>89-93</sup>

Based on histochemical findings at light and electron microscopy, it was hypothesized that common mechanisms may exist for the phenotypic characteristics of skeletal muscle cells in sarcopenia and myotonic dystrophy,<sup>94,95</sup> and a very recent paper confirmed that premature senescence occurs in primary cultures of muscle cells from patients with myotonic dystrophy type 2.<sup>96</sup> It has been demonstrated that an adapted physical exercise has positive effects on the structural features of both skeletal muscle cells and satellite cells:<sup>97-99</sup> Molecular and ultrastructural analyses demonstrated that the myotendinous junctions are also able to positively adapt to stretching forces through the enlargement of the muscle-tendon contact area to increase mechanical resistance.<sup>100,101</sup>

A few papers only have been published on the cytochemical detection of *Cell proliferation & death*,<sup>102-104</sup> whereas it is worth noting that *Regenerative medicine* is presently gaining great attention. Most of the investigations are performed using cultured cells growing onto natural or artificial scaffoldings, where the interactions among grafted cells are essential for allowing growth and differentiation: this makes the analysis *in situ* of morphological, cytokinetic and molecular features essential for assessing the proliferation and reconstructive potential of cells in 2D and 3D cultures.<sup>105-108</sup>

## How the published articles did impact on the biomedical literature

In Figure 2, the impact of the papers published during the last five years was estimated; the articles have been pooled according to the ten categories listed above, and the percentage of citations for each category over the total number of quotations was calculated, for the same time span. As expected, the most cited articles (about 50% of the total) fall within *Human pathology* and *Animal biology & Experimental medicine*, but a significant fraction of citations were for papers on *Methods*, *Development & stem cells* and *Human & comparative histology*.

Looking at the citations received by these articles in 2014 (Figure 3), *Regenerative Medicine* has the highest ratio of citations per published article, confirming that histochemistry may be regarded as a fundamental tool for studying cell interactions with the host tissues or the scaffolding structures, and for describing stem cell proliferation and differentiation. Actually, high ratios were also found

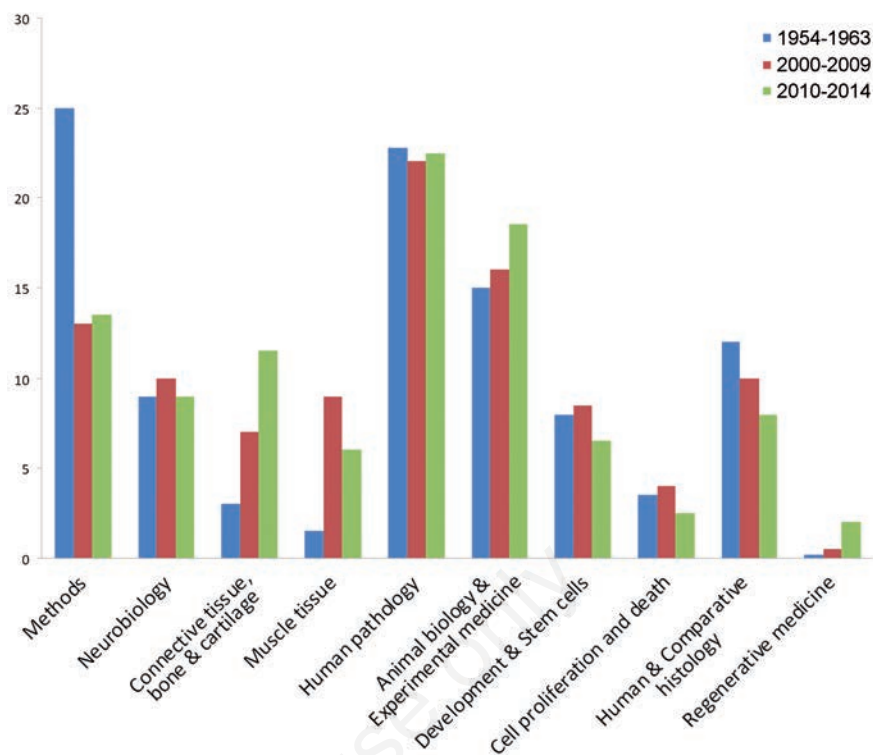


Figure 1. Percentage of articles published in the *European Journal of Histochemistry* in the three time periods, for the different subject categories.

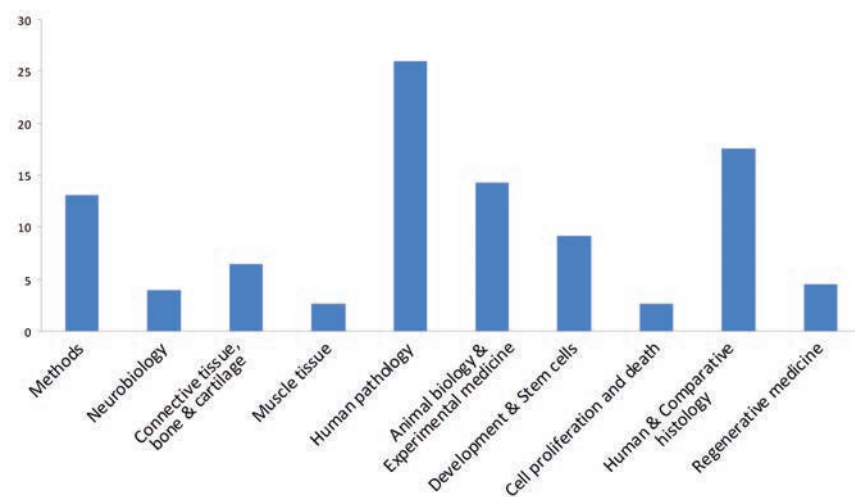


Figure 2. Percentage of citations for the different subject categories over the total number of quotations of the papers published in the *European Journal of Histochemistry*, during the last five years.

for the articles published on *Development & stem cells* and on *Cell proliferation & death*. Unexpectedly, also the articles describing histological features in animal species and humans were highly cited.

To get a further indication of the influence of histochemical investigations on the different fields of biomedical research, in Figure 4 the citing articles of the last five years have been grouped according to the categories of the journals where they have been published, based on the subject categories listed in ISI web of knowledge - Journal Citation Reports.

As expected, most of the citing articles were published in journals of *Microscopy* and *Cell biology*, but the citing journals are scattered over a variety of categories, from *Biochemistry & Molecular biology* to *Reproductive biology*, to *Oncology* and a series of specialized medical subjects, to *Cell & tissue engineering*.

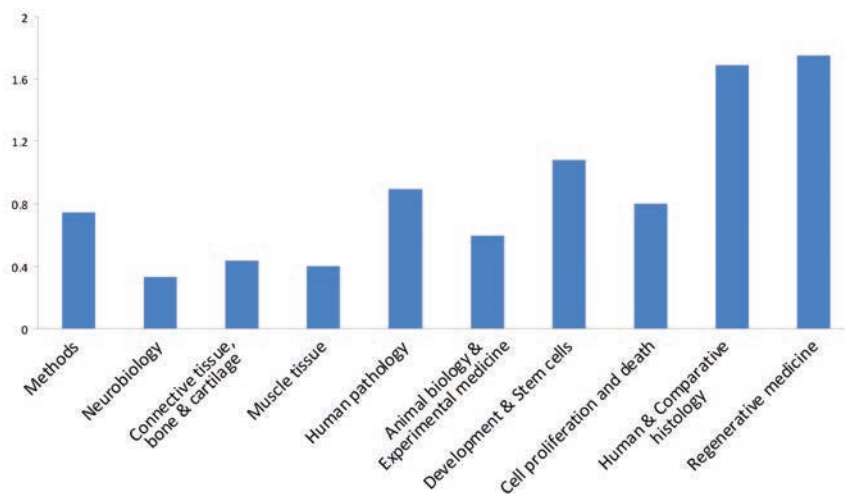


Figure 3. Percentage of citations received in 2014 by the articles published during the last five years in the *European Journal of Histochemistry*, considered per subject category.



Figure 4. Percentage of articles citing the papers published in the *European Journal of Histochemistry* during the last five years; the citing articles have been grouped according to the subject categories of the journals where they have been published (see ISI web of knowledge - Journal Citation Reports).

## Concluding remarks

Histochemistry provides the unique opportunity to detect single molecules in the very place where they exert their structural roles or functional activities. This possibility to correlate structural organization and function may be fruitfully exploited in countless research subjects. Microscopy and histochemistry are appropriate tools for making correlations between microanatomy and physiology in tissues and organs, and the evidence obtained may be suitable for detecting pathological changes as well as for describing still poorly known organisms. A growing body of data showed that not only the expression of specific gene products but also their proper intracellular distribution is essential for the correct function of cells and tissues; again, histochemistry is appropriate for detecting ectopic relocation of molecular components, which often has diagnostic or predictive implications.

This limited survey confirms that histochemical journal, especially when open to a large spectrum of research subjects, do represent a matchless source of information for scientists in manifold research fields of biology and medicine. This was actually expected for cell biologists, microscopists or anatomists, but it is remarkable (though not surprising) that also biochemists, molecular biologists and biotechnologists do find suitable information in histochemical journals.

No doubt, Histochemistry nowadays is still *live and in color*.<sup>109</sup>

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