Early Electron-Microscopy laboratory attendance as an efficacious way of introducing medical students to scientific research

M. Relucenti, E. Battaglione, S. Miglietta, L. Petruzziello and G. Familiari

Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Laboratory of Electron Microscopy "Pietro M. Motta", Sapienza University of Rome, Rome, Italy.

The medical and surgery degree-course at Rome's *Sapienza* University requires that an education in scientific method form a part of a substantially longitudinal approach to its integrated curriculum in "medical and scientific methodology"; this, in the light of the importance "*evidence-based medicine*" has assumed in recent years (1). In order provide (on an elective basis) first-year medical-degree students with early exposure to the reality of work in an ultra-structural research laboratory, we decided to offer them the chance of attending the "Pietro M. Motta" Electron Microscopy laboratory, in small groups. This elective activity was held between March and the end of June 2013; of the 120 students who applied, only those who passed their anatomy oral at the first 2013 exam session were admitted, for a total of 48. The students were divided into 12 groups (4 students per group) and attended the laboratory from Monday to Friday. At the end of this period, all the students involved answered an anonymous questionnaire aimed at assessing the educational usefulness of the experience; 5 students asked to extend the period of the laboratory experience and, as a result, were admitted to active research projects.

The small group of students was guided and supported by a qualified researcher, a post-graduate student, a graduate student and a technical-staff unit. On the first day, the group visited the laboratory where the technicalities of the equipment were illustrated, a lecture on the methods used to prepare the biological samples for Scanning and Transmission Electron Microscopy held, and a procedure for the preparation of samples for Scanning and Transmission Electron Microscopy begun. On the second and third days, the preparation of the samples was completed and a guided discussion, led by the researcher, was held on issues relating to scientific articles concerning the samples being used in the experiments conducted in the laboratory (2,3). On the fourth and fifth days some semi-thin and ultra-thin samples were cut and observed using an optical and an electronic transmission microscope. The samples prepared for the scanning electron microscope were dehydrated, coated and observed through that microscope. At the end of the week, each student had not only observed, but also taken hands-on part in various stages of the preparation and analysis of the samples. The discussion with the researcher and the post-graduate doctors/students provided key concepts regarding scientific widely shared by the students, upon which to base and practice an ultra-structural type of biomedical research project.

The students' early exposure to the work and methodology characteristic of ultra-structural research may prove useful, not only when promoting in-depth understanding of microscopic anatomy, but also as a motivational core into which instill a correct approach to scientific research in future doctors.

Keywords: electron microscopy; medical education; learning by doing; active learning

1. Introduction and purpose of the work

The medical and surgery degree-course at Rome's *La Sapienza* University requires that a sound education in scientific methods be part of a substantially longitudinal approach to its six-year integrated course in "medical and scientific methodology". During the first year, the students enrolled in the course are required to assimilate basic concepts concerning the evolution of medicine down through the centuries (the evolution of medical thinking). In particular they are expected to acquire a thorough knowledge of the conceptual structure of medicine and the philosophy of the natural sciences, with particular reference to scientific and non-scientific knowledge, the birth and characteristics of modern science, the scientific method, facts and hypotheses, the laws of science, objectivity, scientific error, proof and falsification accruing to scientific elucidations. In the light of the importance that "evidence-based medicine" has acquired in recent years [1], it has been deemed important to provide students with early exposure to the reality of work in an ultra-structural research laboratory. The students interested in this experiment were enrolled in the second semester of the first year of our medical school.

Our university's medicine and surgery degree courses, foresee that the students attending the final three years follow a "path of excellence." This integrative course consists in a series of educational activities additional to those on the students' ordinary syllabi for those years. The path of excellence includes participation in interdisciplinary, educational activities such as seminars and training practices (a maximum of 200 hours per annum). These activities are aimed at implementing guided research projects led by and in cooperation with faculty mentors, clinical and/or laboratory staff [2].

2. Materials and methods

The elective course in question was held at the "Pietro Motta" laboratory of electron microscopy, Department of Anatomical, Histological, Forensic and Orthopaedic Sciences at the *Sapienza* University of Rome, Italy. Attendance at the laboratory began in the month of March 2013 and continued until the end of June 2013. During the months of January and February requests for attendance (N=120), were received from 120 of the faculty's first year students; of these only those who had passed the first 2013 session's human anatomy and clinical medicine 1 examinations (N= 48 students) were accepted. These students were divided into groups of 4 and attended the laboratory from 9.00 am to 5.00 pm for five consecutive days, from Monday to Friday. The groups of students were taught and supported by a team of one researcher, one PhD Student, acting as tutors as well as by a team of technicians.

2.1 The first day

The students were shown around the various premises of the laboratory, and in each one of these they were introduced to the various apparatus and received explanations regarding the laboratory's general working principles. At the end of this visit the tutors held a seminar on the ways in which biological samples are prepared for scanning and transmission electron microscopes. During the seminar, particular emphasis was placed on the laboratory's health and safety systems and the correct procedures to follow when treating and handling biological samples. At the end of the seminar the students were given copies of the slides used to permit them to bring informative material home with them.
Then, the procedure by which a number of endarterectomized carotid samples are prepared for scanning and transmission electronic microscopy was explained and applied. The students, under the guidance of their tutors:

- prepared glutaraldehyde solutions,
- prepared the identification tags to the test tubes ,
- prepared PBS swabs,
- rinsed the fixed samples.

The post-fixation in osmium tetroxide and subsequent washings were carried out by the tutor.
The students, under the guidance of their tutors, prepared the 30-50-70% alcohol solutions and carried out the dehydration of the samples.
The samples were left overnight at 4°C.

2.2 The second day

In relation to the preparation of the samples for transmission electron microscopy examination, the students, under the guidance of their tutors:

- prepared the 95 and 100% alcohol solutions and carried out the dehydration of the samples,
- prepared the epoxy resin used to place the samples in the transmission electron microscopy,
- placed the samples in propylene oxide to facilitate the substitution procedure.

Having left the samples to rest overnight in a solution of 50% propylene oxide and epoxy resin.

In relation to the preparation of the samples to be examined availing of scanning electron microscopy, the students, under the guidance of their tutors:

- dehydrated the samples using an EMITECH K850, "critical point drying" apparatus,
- stuck the dehydrated samples onto the aluminum stubs with silver glue.

2.3 The third day

In relation to the preparation of the samples for transmission electron microscopy examination, the students, under the guidance of their tutors:

- prepared the identification tags for the samples,
- placed the samples in their capsules with their respective tags,
- added the epoxy resin,
- placed the samples in a stove at 60°C.

In relation to the preparation of the samples for scanning electron microscopic examination, the students metallized some samples using an EMITECH K 550 sputter coater.
They examined these samples on a Hitachi S400 field emission scanning electron microscope, interfaced with a DISS 5 Point Electronic imaging and analysis system.

2.4 The fourth day

A researcher-led reading of scientific articles concerning samples like those used in the laboratory experiments [3, 4] was carried out. The images obtained the day before, using the scanning electron microscope, and the measurements thus obtained, were appraised availing of statistical methodologies with the aid of Med Calc software.

2.5 The fifth day

Some semi-thin sections were cut availing of a LEICA EMUC6 ultra-microtome, stained with methylene blue and observed, first by light microscopy using a Zeiss Axioskop 40 optic microscope. Then, ultra-thin sections were cut and stained for examination under a Zeiss EM 10 transmission electron microscope. These procedures were performed by the tutor and observed by the students.

At the end of this experience all the students anonymously answered a questionnaire aimed at obtaining an appraisal of the didactic usefulness of these laboratory practices.

3. Results

The main results obtained through the anonymous questionnaires are analytically reported in tables 1-5.

The results reported in table 1 show how the rich iconography availed of during the microscopic anatomy lessons encouraged the students to attend the electron microscopy laboratory and secondly highlighted the curiosity the first-year students showed towards activities carried out in the laboratory. Only a small number of the students showed an interest in pursuing a career in research.

Table 1 Student motivation for laboratory experience

Statement	%
The electron microscopy images viewed during class intrigued me (N= 20) /48	41,67
I had never entered into a research lab and I was curious to see how it worked (N=17) /48	35,41
Attending a laboratory workshop is more useful than going to class (N=5) /48	10,42
I am interested in laboratory work, in the future I would like to be a researcher (N=6) /48	12,5
No answer (N=0) /48	0

The results contained in table 2 show that the students grasped the following fully: the importance of identifying samples properly; the usefulness of contextual completion of worksheets to achieve the reproducibility of future results; the importance of accuracy in the preparation of the solutions used. As the purpose of this part of the practical experience goes beyond any single experiment, our intention was, in fact, that of having the students understand the importance of being methodical and precise, a concept they may easily translate into clinical practice, where the same method and care must be applied when compiling a medical record or administering drugs.

Table 2 Sample preparation procedure

It is important to pay attention during the identification of the samples to avoid confusing them	Average (± sd)	95% CI for the average	Mode	Cronbach alpha
The identification of the samples is not vital	4.1667 ± 0.3766	4.0573-4.2760	4	0.8424
It is important to fill out the worksheets during experiments to strictly follow the established protocol	1.3333±0.4764	1.1950-1.4717	1	0.8300
It is useless to fill out the worksheet during the experiments as things are done in the same way	4.3958±0.4942	4.2523-4.5393	4	0.8344
For the success of experiments the solutions used need to be prepared with extreme precision	1.8542±0.5049	1.7076-2.0008	2	0.8537
Accurate preparation of the solutions used does not affect the success of the experiment	4.2292±0.4247	4.1058-4.3525	4	0.8340
Following a protocol favors obtaining reproducible results	1.7500±0.43	1.6229-1.8771	2	0.8598
Following a protocol and reproducible results unrelated factors	4.0417±0.2019	3.9830-4.1003	4	0.8652
It is important to pay attention during the identification of the samples to avoid confusing them	2.0208±0.2518	1.9477-2.0939	2	0.8620

Likert scale: 1= Strongly disagree, 2= Disagree, 3= Neither agree nor disagree, 4= Agree, 5= Strongly agree. Cronbach alpha= 0.8651

The results reported in table 3 regard the observations, availing of optical, scanning and transmission electron microscopes, carried out on the samples examined during the practices. The aim of this part of the experience was that of acquiring specifically anatomical as well as generally translational skills. The results show that the observations carried out on the samples using optical, scanning and transmission electron microscopes permitted the students to understand the true form of cells and the relative dimensions of the organelles contained in them.

Direct observational activity should enable students to make conscious choices regarding the microscopic technique best suited to a certain kind of observation of a given sample, and know whether to opt for a scanning or transmission electron microscope, depending on the kind of sample to be appraised.

The translational skills acquired concern the subsequent elaboration of images. During this phase of the activity the students used and examined software availed of to analyze images and observed the application of statistical analysis to the results obtained. This showed how the knowledge of statistics acquired during their physics and scientific medical methodology courses find significant practical application in the examination of biological images. These statistical methodologies may be applied later on by the students, for example, in clinical anatomical pathological practice.

Table 3 Sample observation by light microscopy, transmission electron microscopy, scanning electron microscopy

Statement	Average (± sd)	95% CI for the average	Mode	Cronbach alpha
The microscopes must be properly centered for distortion-free images	4.0208±0.3853	3.9089-4.1327	4	0.9036
It suffices to turn on the microscope to begin observation	2.2708±0.6438	2.0839-2.4578	2	0.9115
The transmission electron microscope permits one to see cell organelles like the mitochondria Golgi apparatus, etc.	4.0625±0.2446	3.9915-4.1335	4	0.9153
Optical microscopy too permits one to see cell organelles like the mitochondria Golgi apparatus, etc.	2.0000±0.2917	1.9153-2.0847	2	0.9102
Scanning electron microscopes shows that cell surfaces are not always perfectly smooth, but generally contain pseudopods, microvilli, cilia etc.	4.1250±0.4436	3.9962-4.2538	4	0.9011
The cells that are perfectly spherical, pseudopodia and microvilli are preparatory artifacts	1.9792±0.2518	1.9061-2.0523	2	0.9162
By measuring and processing the results obtained, one favors an understanding of practical applications of statistics	4.1250±0.4436	3.9962-4.2538	4	0.9011
Measuring and processing the results obtained is useless and boring	2.1458±0.4608	2.0120-2.2796	2	0.9062

Likert scale: 1= Strongly disagree, 2= Disagree, 3= Neither agree nor disagree, 4= Agree, 5= Strongly agree. Cronbach alpha= 0.9194

The results shown in table 4 concern the researcher-guided reading of scientific articles. This part of the laboratory experience served, essentially, two purposes; the first was that of making the students aware of the principal bibliographical research engines available within the biomedical field (Pub Med, Google scholar, Scopus) and the differences existing between a truly scientific and a generically divulgation journal which, anyone may purchase from a newsstand, for example. The second aim was that of illustrating the parts that constitute a scientific article, its function, and the differences existing between the parts. During this phase of the laboratory experience the students were reminded of what they were told about compiling worksheets and the importance of filling in the sections regarding materials and methods precisely and correctly. The results reported in this table show the importance of interaction with the researcher during this stage of the practices and how the students understood and learnt during the various phases of this scientific experience.

The results illustrated in table 5 regard the students' overall appraisal of their attendance at the elective electronic microscopy laboratory. The students appreciated considerably their interaction with the Ph. D students and the researchers, considering this contact strongly stimulating. The results show that all the phases of the practices helped the students to begin to form a scientific mind set, attentive to the results obtained by recourse to repeatable methods. They reveal a high degree of satisfaction with regard to the various techniques observed and/or put into practice during these lessons and the chance, during the various phases of their work at the laboratory, of practically applying knowledge acquired while studying the other disciplines (chemistry, physics, microscopic anatomy, statistics, and scientific medical methodology).

Table 4 Researcher guided reading of scientific papers

Statement	Average (± sd)	95% CI for the average	Mode	Cronbach alpha
Guided reading fosters an understanding of the importance of abstracts to scientific papers	4.0417±0.2887	3.9578-4.1255	4	0.9135
Abstracts are mere summaries, it is best to read the entire article	2.2708±0.9165	2.0047-2.5370	2	0.9085
Guided readings permit us to understand the role of introductions	4.1458±0.4608	4.0120-4.2796	4	0.9002
One may safely avoid reading introductions	1.7708±0.4722	1.6337-1.9079	2	0.9056
Guided reading helps us understand the importance of keeping a protocol during the experiments when writing up the materials and methods section	4.3125±0.4684	4.1765-4.4485	4	0.9018
It is useless to re-read protocols when writing a work, methods and materials do not need to be written in depth	1.7083±0.4593	1.575-1.8417	2	0.9084
Interaction with a researcher helps us understand why exposition of results must precede discussion and how these two parts of scientific work differ	4.3333±0.5191	4.1826-4.4841	4	0.8977
It is useless to have the investigator matters, results and discussion are the same thing	1.9583±0.3548	1.8553-2.0614	2	0.9092
Interaction with the researcher provides insight into how to set up a discussion and how to report the conclusions of work in a concise but comprehensive manner.	4.3958±0.5355	4.2403-4.5513	4	0.8994
It is useless to have things explained by the investigator, as results and conclusions are the same thing	1.937±0.3200	1.8446-2.0304	2	0.9118

Likert scale: 1= Strongly disagree, 2= Disagree, 3= Neither agree nor disagree, 4= Agree, 5= Strongly agree. Cronbach alpha=0.9144

Table 5 Overall judgment on laboratory experience

Statement	Average (± sd)	95% CI for the average	Mode	Cronbach alpha
These elective practices in the electron microscopy laboratory permitted me to put into practice basic concepts concerning the other disciplines taught during the first year of the course: chemistry, physics, microscopic anatomy, statistics, medical and scientific methodology.	4.3750±0.4892	4.2329-4.5171	4	0.9093
These basic concepts are useless to a doctor, they are of use to researchers only	1.8333±0.5586	1.6711-1.9955	2	0.9046
These elective practices in the electron microscopy laboratory show that it is not necessary to pay attention while carrying out experiments	1.8333±0.4292	1.7086-1.9580	2	0.8959
These elective practices in the electron microscopy laboratory help understand that in order to make a statement in science one needs the support of evidence.	4.1250±0.3928	4.0110-4.2390	4	0.9029
Elective practices in an electron microscopy laboratory is not useful to the acquisition of a scientific method	1.7708±0.4247	1.6475-1.8942	2	0.9069
Elective practices in a electron microscopy laboratory permits the acquisition of many laboratory techniques	4.1042±0.5550	3.9430-4.2653	4	0.9177
During elective practices an electron microscopy laboratory does not teach laboratory techniques	1.8333±0.3766	1.7240-1.9427	2	0.9160
Elective practices in a electron microscopy laboratory permits interaction with Ph. D students and researchers, this receiving precious stimuli	4.2500±0.4838	4.1095-4.3905	4	0.8915
Elective practices in an electron microscopy laboratory obliges interaction with Ph. D students and researchers, which embarrasses the students	1.5833±0.5392	1.4268-1.7399	2	0.9059

Likert scale: 1= Strongly disagree, 2= Disagree, 3= Neither agree nor disagree, 4= Agree, 5= Strongly agree. Cronbach alpha=0.9154

4. Discussion and Conclusions

As "evidence-based medicine" has grown increasingly important in recent years, medical and scientific methodologies are taught in this faculty during an integrated longitudinal course spanning six years. Only students attending the final three years of the degree course are involved in a "path of excellence", which permits them to take direct part in research projects under the guidance and with the cooperation of faculty mentors. Several studies have yielded data

concerning the benefits to undergraduate students of participation in research, of blending teaching and research, of approaching primary literature [5,6,7]. We felt that practical training in an electron microscopy laboratory might turn out to be a fruitful way of introducing students to the world of research and of bringing the scientific method home to them in a direct, "hands-on" manner.

In fact, processing a sample for examination under an electron microscope requires several days' preparation and the use of different instruments, implies a sound knowledge of chemistry and physics. These factors make practices in an electron microscopy laboratory committing and complete, particularly in the case of students attending the first year of medical school. Practice in the use of electronic microscopes permits students to understand the importance of methodicality and precision, it also permits them to understand the true structure of cells and the relative dimensions of the organelles contained in them. Direct observational experience enables the students to make informed choices regarding the elective electron microscopic technique to choose in order to examine a given sample. A knowledge of the statistical methods acquired during their physics and scientific medical methodology courses find significant and meaningful application during the analyses of the biological images acquired during the laboratory practices. These statistical methods may also be applied by the students during the practice of anatomical pathology, for example. Their interaction with the Ph. D students and the technical staff during the various phases of the experience also contributed towards producing a scientific mind set in the students, making them attentive towards the results obtained by the applications of repeatable methodologies. A high degree of satisfaction was shown concerning understanding the different functions of the various phases of scientific work; the diverse techniques observed and/or put into practice during attendance; the chance of putting into real practice during the various stages of the practices of skills acquired while studying the other disciplines on their course (chemistry, physics, Microscopic anatomy, statistics, scientific medical methodology), are all factors that show how early attendance at an electronic-microscope laboratory can prove an efficacious means of introducing medical students to scientific research. The primary mission of a scientific university degree is not simply to carry out research but also to introduce students to research first though observation, then by active participation in research. This is the only way a passion for discovery can be inspired.

References

[1] Snelgrove H, Familiari G, Gallo P, Gaudio E, Lenzi A, Ziparo V, Frati L. The challenge of reform: 10 years of curricula change in Italian medical schools. Med Teach. 2009 Dec;31(12):1047-55.

[2] Salvetti M, Palmieri G, Grassi F, Pontieri F, Stoppacciaro A, Di Somma S, Familiari G, Ziparo V. Teaching research methodology in the medical under graduate curriculum at Sapienza University of Rome: a two-year experienced model. Congress of the Association for Medical Education in Europe (AMEE), Malaga, Spain, 27 August – 3September 2009, Abstract Book.

[3] Familiari G, Heyn R, Relucenti M, Nottola SA, Sathananthan AH. Ultrastructural dynamics of human reproduction, from ovulation to fertilization and early embryo development. Int Rev Cytol. 2006;249:53-141.

[4] Relucenti M, Heyn R, Petruzziello L, Pugliese G, Taurino M, Familiari G. Detecting microcalcifications in atherosclerotic plaques by a simple trichromic staining method for epoxy embedded carotid endarterectomies. Eur J Histochem. 2010 Jul 14;54(3):e33.

[5] Gregg-Jolly LA, Kington R, Lopatto D, Swartz JE. Benefits of intertwining teaching and research. Science. 2011 Feb 4;331:532.

[6] Hoskins SG, Lopatto D, Stevens LM. The C.R.E.A.T.E. approach to primary literature shifts undergraduates' self-assessed ability to read and analyze journal articles, attitudes about science, and epistemological beliefs.CBE Life Sci Educ. 2011;10(4):368-78. .

[7] Lopatto D. Undergraduate research experiences support science career decisions and active learning.CBE Life Sci Educ. 2007;6(4):297-306

