Teaching Cell Biology to Dental Students with a Project-Based Learning Approach

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Abstract: Although the discipline of cell biology (CB) is part of the curricula of predoctoral dental schools, students often fail to recognize its practical relevance. The aim of this study was to assess the effectiveness of a practical-theoretical project-based course in closing the gaps among CB, scientific research, and dentistry for dental students. A project-based learning course was developed with nine sequential lessons to evaluate 108 undergraduate dental students enrolled in CB classes of a Brazilian school of dentistry during 2013-16. To highlight the relevance of in vitro studies in the preclinical evaluation of dental materials at the cellular level, the students were challenged to complete the process of drafting a protocol and performing a cytocompatibility assay for a bone substitute used in dentistry. Class activities included small group discussions, scientific database search and article presentations, protocol development, lab experimentation, and writing of a final scientific report. A control group of 31 students attended only one laboratory class on the same theme, and the final reports were compared between the two groups. The results showed that the project-based learning students had superior outcomes in acknowledging the relevance of in vitro methods during biocompatibility testing. Moreover, they produced scientifically sound reports with more content on methodological issues, the relationship with dentistry, and the scientific literature than the control group (p<0.05). The project-based learning students also recognized a higher relevance of scientific research and CB to dental practice. These results suggest that a project-based approach can help contextualize scientific research in dental curricula.

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CB) is a typical discipline in dental school curricula that contributes to the understanding of basic concepts in health sciences and is applied in translational research.¹ However, the teaching of CB remains mostly dependent on conventional visual-descriptive strategies² that have been criticized for having low student participation,³ reduced contribution for critical thinking or practical skills development, and not acknowledging the practical relevance of basic disciplines.⁴ Consequently, a decade after Iacopino emphasized the relevance of scientific subjects such as cell/molecular biology, genetics, and tissue engineering to dentistry,⁵ scientific and technological knowledge still finds limited permeation into dental curricula.⁶

Delaying the introduction to project development and scientific thinking in dental schools can compromise the ability of students to make the practical links between the early basic biology classes and the clinical practice.⁷ Indeed, basic science teaching has often been characterized by extensive contents and factual subjects disconnected from clinical relevance and practical applications.^{8,9} Studies have found that lack of institutional incentives, inadequate infrastructure, insufficient time, and increased class sizes, as well as the limited involvement of faculty with research activities, were substantial barriers to the diffusion of basic and research-related knowledge on dental education.^{5,10} Also, broad use of the "2 years of basic sciences + 2 years of clinical training" format in dental curricula, with the basic disciplines offered mostly by departments or faculty members not directly involved with the dental school, may contribute to the fragmentation of knowledge, education that is not patient-centered, and the disconnection of subjects such as cell/molecular biology from their clinical relevance ¹¹

On the other hand, initiatives have arisen prioritizing an active and integrated learning from basic science to clinical content,¹²⁻¹⁵ including meth-

odologies such as problem-based learning (PBL) that support integration among courses and disciplines.¹⁶ Nevertheless, in settings where there is no institutional policy for an integrated interdisciplinary approach, individual initiatives can be the bridge to a structural change, promoting the use of directed case studies,¹⁷ case-based learning,^{11,18} team-based learning,¹⁹ and interactive learning through games,²⁰ among the emerging methodologies. In this regard, Hendricson et al. provided an overview of successful educational strategies associated with the development of problem-solving, critical thinking, and self-directed learning.²¹

Laboratory classes also impact the development of practical and professional skills and contribute to the better understanding of biological processes.²² Likewise, institutional strategies, such as implementing compulsory research for students, might contribute to the scientific development of the dental profession.²³ Another promising approach in higher education is project-based learning,²⁴ which has some of the same benefits as PBL. According to Kolmos and Graaff as well as Hanney and Savin-Baden, PBL may be adapted to different educational contexts, enabling the use of project management tools to structure a learning exercise.^{25,26} Moreover, when linked to research and laboratorial activities, project-based learning may improve the attitudes of dental students about science and impact their choice of academic/research-related professional activities after graduation as advocated by Rosenstiel and Johnston²⁷ or at least form dentists who are "sophisticated consumers of research" in the words of Iacopino⁵ and able to make evidence-based decisions and use scientifically proven methods/materials in their clinical practice. The aim of this study was thus to assess the effectiveness of a practical-theoretical project-based course in closing the gaps among CB, scientific research, and dentistry for dental students. We sought to address the search for an active education methodology and increase the contribution of CB teaching to the professional development of dental students as well as their scientific knowledge acquisition.

Methods

This study was approved by the Research Ethics Committee of Fluminense Federal University, Niteroi, Brazil (CEP-HUAP-UFF, approval CAAE:53301816.1.0000.5243). The project-based learning intervention was implemented during the regular CB course at Fluminense Federal University School of Dentistry, with 138 students from four classes between 2013 and 2016. Classes were led by two professors and assisted by three graduate students as tutors. Scientific and academic background information of a representative sample of the students (n=37) was collected in the first week through a questionnaire assessing their basic science research or laboratory experience.

A control group of 27 students from a class that did not participate in project-based learning had a single laboratory class in which they performed the same cytotoxicity test proposed for the project-based learning group. Afterwards, students had to submit a final report, which was used as a control for the learning outcome. A survey on the relevance of CB was also answered by 31 students from another class that did not perform any laboratory activity. A final assessment was performed with the project-based learning students (n=30) four years after the intervention to assess the retention of scientific knowledge acquired by the intervention. All activities were performed in a total of 20 hours distributed in nine lessons and divided into five major steps.

A two-item questionnaire on basic concepts of in vitro dental biomaterial testing was distributed at the end of the course. A semi-quantitative survey assessing self-learning, perception of weak and strong points of the methodology, and applicability of CB in dental education was also answered anonymously at the end of the course by all participating students. Students, in groups of three to five, also had to submit a final report on the project.

Discourse analysis of the structure and contents of the questionnaires and of the final reports was performed. The content related to the scientific method, to elements of critical thinking,²⁸ or to the relevance of CB in dentistry was organized into categories and tabulated. The total ratios of answers in each category were compared for the project-based learning and non-project-based learning cohorts by the chi-square test, at 95% significance, with the software GraphPad Prisma 6.0 (GraphPad Inc., San Diego, CA, USA).

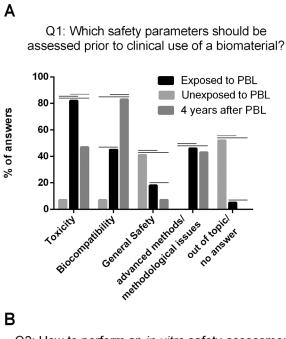
Results

On the survey of students' scientific/academic background prior to the project-based learning intervention, most students (93%) had no previous laboratory experience. Also, 85% of the students had never heard of scientific databases, and 59% had never read a peer-reviewed scientific article. None of them had produced a science report, and 66% had never given a seminar or an oral presentation in class prior to the beginning of the course.

After the introduction to the proposed research theme (Table 1, Step I), the project-based learning students were already able to identify the main parameters that should be evaluated before the clinical approval of dental materials (Figure 1, panel A). Among these students, 46% provided answers that included the use of advanced assays (e.g., genotoxicity, cell differentiation) and methodological issues such as the use of controls and blanks. Four years after the intervention, the concept of biocompatibility was significantly more present in their answers (p<0.05). On the other hand, the students who did not participate in project-based learning provided significantly different answers (p<0.05), as most failed to answer or stated that a "general risk assessment" should be performed without further explanation.

Lesson Number	Time Load	Setting	Content
Step I: Presentation	n of initial scena	rio and background	
1	120 min	Classroom	 Introduction to project proposal: biocompatibility assay of dental biomaterials. Reading and discussion of book chapter on biomaterials in dentistry. Division of students into groups of 3 to 5.
Step II: Scientific d	atabase search		
2	120 min	Classroom/ computer lab	 Questionnaire application with two questions on basic concepts of in vitro biomaterial testing. Search in scientific databases. Selection of research articles on in vitro biocompatibility testing.
3	120 min	Classroom	1. Article presentation in format of seminars.
Step III: Exposure t	o a research lab	oratory environment	
4	240 min	Research lab	1. Visit to research laboratory specialized in toxicological analysis of medical and dental materials.
Step IV: Experimen	ıt		
5	120 min	Lab	 Presentation of lab infrastructure and biosafety principals. Preparation of solutions for in vitro assays (e.g., culture media and buffers).
6	120 min	Classroom	1. Preparing the assay protocol: students received biomaterial samples (synthetic hydroxyapatite spheres) and were invited to develop a feasible protocol for a cell viability assay to be performed during a lab class (up to 4 hours) using the available infrastructure. Professors acted as mediators, addressing questions and listing available materials. All conflicting ideas were submitted to voting.
7	240 min	Lab	 Cytocompatibility test performed according to the protocol developed by students.
Step V: Data interp	retation and rep	ort	
8	120 min	Classroom/ computer lab	 Tabulation of results on Excel spreadsheet; basic treatment of data, including data reductions, data normalization, calculation of means and standard deviations, and plotting graphical representations of results. Brief (30 min) theoretical presentation on the production of scientific reports. Each group had 2 to 3 weeks to submit the final report, consisting of introduction, methods, results, discussion, and bibliography.
9	60 min	Classroom	 Submission of final report. Anonymous assessment of students' attitudes toward cell biology and the course in general.

Table 1. Structure of theoretical and practical lessons of the project-based course



Q2: How to perform an *in vitro* safety assessment of a dental material?

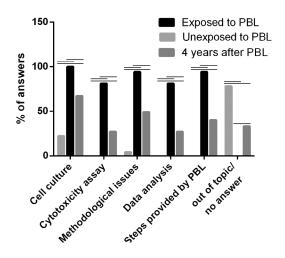


Figure 1. Comparison of students' percentages of correct answers for those in project-based learning, not in project-based learning, and four years after project-based learning

Note: In labels for this figure, "PBL" refers to project-based learning.

The concepts acquired in Step I served as the basis for an online search for original articles on the in vitro biocompatibility evaluation of biomaterials (Step II). This process allowed for an effective selection of scientific studies, and 87% (n=25) of the students used methodologies that could indeed be

replicated in our laboratory. The seminar presentations at the end of Step II were strongly focused on methodologies, interpretation of results, and critical reading of the scientific literature. In general, students had difficulty understanding the methodological descriptions of laboratory analyses, which was probably due to their lack of previous contact with this type of environment. Such difficulty, however, appeared to be overcome by visiting a toxicological testing laboratory, where students had the opportunity to see and "touch" the actual procedures and ask further questions on cell culture, reagents, and equipment involved in dental material testing (Step III).

Information from the seminars and from the lab visit was used in the discussion of the material testing protocol development (Step IV; Table 2). At least one group in each class suggested the adherence to International Standards when deciding which methodology to use, and most groups chose to test cell types that were applied in the studies from the seminars. All groups and classes remembered to include controls (both positive and negative), validated the assays, and performed replicate analysis, often citing the seminar papers. Some classes included the use of dilution curves of the material extracts or increasing exposure times.

Performing the assays was another opportunity for students to get hands-on contact with laboratory activities. Groups were encouraged to operate with task division (e.g., each member being responsible for a different step of the protocol). During the practical work, students participated actively and with focus, were concerned with the quality of their results, and declared themselves "proud" of using their own manufactured reagents.

Most students had their first contact with an Excel spreadsheet, data reduction, and scientific representations during the subsequent data analysis and results discussion (Step V). Most groups chose to combine their data as replicates of the same experiment, decreasing the standard deviation of the final results for the whole class.

Students who participated in the practical course presented slightly higher mean grades on the overall CB course compared to students who attended only the regular (theoretical) course (7.8 ± 1.2 and 7.2 ± 1.6 , respectively, on a 0-10 scale). Nevertheless, the expected learning outcome from this project-based learning activity related more to scientific skills than theoretical knowledge. Therefore, the project-based learning outcome was more adequately assessed by comparing the final scientific reports

Protocol Parameter	Choice Example	Main Source and Reason for Students' Choices
Cell model	Human primary osteoblasts	Cell model should emulate the target tissue of bone regeneration; the use of primary human cells aims to reduce estimation from transformed animal cells.
Cell density	10,000 cells per well of a cell culture plate	Most studies selected for the seminars employed a similar cell density.
Type of exposure	Indirect: cells would be exposed to conditioned media that had been exposed to the material to be tested	Seminars presented data of increased sensitivity for indirect methods.
Preparation of conditioned media	200 mg of biomaterial/mL of cell culture media were extracted at 37°C/24 h	Groups proposed the use of the international standard (ISO 7405:2008) guidelines
Time of exposure	Cells would be exposed to 24 h, 48 h, or 72 h	Students decided to assess the effect of time of extraction on the cytotoxicity of a material.
Replicates	Two technical replicates per group were performed; all groups provided technical replicates for each other	Aiming for more reliable results, with decreased standard deviation.
Use of controls	Latex as positive control; polystyrene beads as negative control; unexposed cells as experimental control	The use of positive and negative controls was repeatedly discussed on the seminars. The choice of materials was based on their availability in the laboratory.
Cell viability assay	Crystal violet dye exclusion (density evaluation of viable cells by DNA staining)	Choice based on shorter incubation times, aiming to leave more time for other experimental procedures.

Table 2. Typical structure of a protocol developed by students for material testing step
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produced by the project-based learning students with those from students who only attended a single laboratory lesson.

Students were evaluated for their use of main scientific concepts and their knowledge of the theme in the final reports (Table 3). Important topics and contents covered in the steps of the course (e.g., connection with the dental practice, use of standardized protocols, internal validation by using controls) were significantly more discussed (p<0.05) in the reports from the project-based learning students than those who attended a single laboratory class. Most project-based learning groups discussed the relevance of biomaterials testing in the dental field, setting clear and concise objectives for the solution of the initial problem and reaching matching conclusions supported by results.

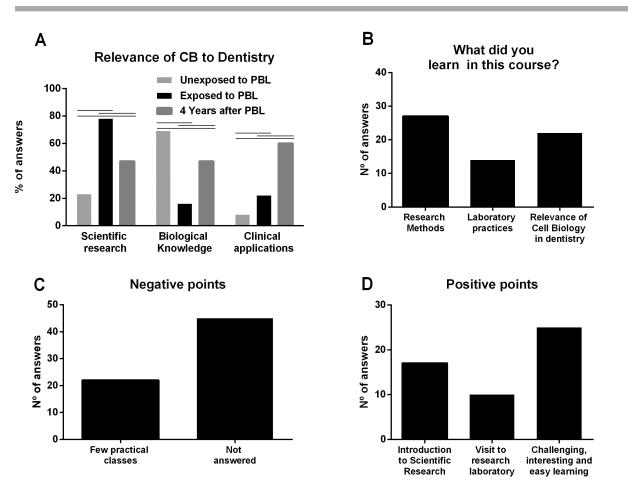
Significant differences (p<0.05) were also found in individual answers to "how to perform an in vitro safety assessment of a dental material" between students from the project-based learning course and those who did not participate in the course and laboratory classes by the end of the CB course (Figure 1, panel B). Concepts such as cytotoxicity assays and methodological issues (e.g., replicates, controls) were almost exclusively found in the answers from the project-based learning students, while the majority (78%) of the other students failed to provide an adequate answer and a lower proportion (22%) simply proposed the use of cell testing without further details. Four years after the intervention, correct answers (69%) presented the previously identified scientific and methodological content, however at intermediary rates that were significantly different from those provided immediately after the course or from the non-project-based learning students (p<0.05).

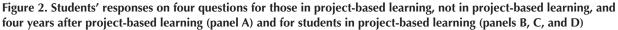
In the assessment of students' attitudes towards CB, the majority (95% of project-based learning students, n=67; 97% of non-project-based learning students, n=31) answered that they could see its applications in dentistry. However, when asked to identify such applications (Figure 2, panel A), the most frequent category of answers changed from "understanding of the biological basis of dentistry" of the non-project-based learning students to "scientific research related to dentistry" of the project-based learning group, including statements mostly related to biomaterials and dental materials (64%). Also, a higher proportion of project-based learning students (p<0.05) recognized clinical applications

Report Topic/Content	Example of Statements	Number (%) of Reports (No Project-Based Learning)	Number (%) of Reports (Project-Based Learning)
Biomaterial definitions and relevance	"Hydroxyapatite is a natural mineral found in bones and teeth with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Because it represents 30 to 70% of the mass of bones and teeth"	10/16 (63%)	20/25 (80%)
Connection with dentistry	"There is a great interest in the production of synthetic biomaterials for bone replacement in dental implantology and prosthodontics." "The use of this material can be broad, from bone grafting processes to esthetic or non-esthet dental procedures."	7/16 (44%) tic	19/25 (76%)
Relevance of material testing	"However, all material to be used in humans must first go through tests that evaluate if it is biocompatible, that is, if it is not harmful to the organism. The procedures to be implemente for the biocompatibility assessments are genotoxicity, cytotoxicity, exposure dose, and others that indicate if the material can or cannot be implanted and if there is any restriction in its us	t	23/25 (92%)
In vitro assays	"In this way, another possibility that has been widely considered are tests involving cell cultur and equivalent tissue models. In vitro tests offer both economic and ethical advantages when compared to in vivo tests."	11/16 e (69%)	11/25 (44%)
Clear and concise objectives	"This study aimed to evaluate the in vitro biocompatibility of hydroxyapatite spheres with murine pre-osteoblasts."	9/16 (56%)	20/25 (80%)
Use of international standards	"The cytotoxicity assay was performed accordin to the guidelines of ISO 10993-5:2009."	g 3/16 (19%)	16/25 (64%)
Comparison with data from literature	"The positive control behaved as expected, sinc latex fragments have been previously proven to be highly cytotoxic (Lourenço et al., 2015)."	e 5/16 (31%)	12/25 (48%)
Internal validation by use of controls	"The graph shows that the negative control group treated with polystyrene, which is biocompatible, kept all the cells alive and that the positive control, treated with latex, which is not biocompatible, obtained 20% of viable cells, as expected. These results validate the experiment."	4/16 (25%)	14/25 (56%)
Other methodological aspects (use of replicates, blanks, statistics)	"The result from the blank group was subtracted from the other wells, removing the unspecific response on wells without any cell and solution "The use of the results of the other groups as replicates allowed the calculation of means with eight replicates, which are more reliable, even though it possibly increased the standard deviation	(31%) ."	18/25 (72%)

Note: Reports were analyzed from students who participated in project-based learning (n=25) and students who participated in a single laboratory lesson with no project-based learning (n=16). There was a significant difference in the content of reports between groups according to chi-square test (p<0.05).

for CB, such as the use of mesenchymal stem cells during cell therapy or the in vitro preclinical trials for dental materials. In the four-year assessment, the mean percentage of biological and research aspects of CB attained intermediate levels, significantly different for the answers of project-based learning and non-project-based learning students (p<0.05). Also, answers included higher levels of clinical applications for CB content (60% versus 22%, p<0.05), probably due to acquired knowledge on dental clinics from other disciplines.





Note: In labels for this figure, "PBL" refers to project-based learning.

In the self-learning evaluation after projectbased learning, students reported a better understanding of research and laboratory-related activities, pointing to the relevance and applicability of CB in dentistry (Figure 2, panel B). Concerning the project weaknesses, 22 students mentioned that the course should have more practical classes (panel C). Regarding its strengths, a positive attitude of students towards this teaching strategy was seen. Students reported that the course was challenging, interesting, facilitated the understanding of both theoretical and practical aspects of CB (panel D), and contributed to their initiation into the scientific world, including statements such as "The CB course presented a broader view of the dental field," "It changed the static idea of the cell," and "It triggered the interest in research."

Discussion

Cell biology is usually presented to dental students as a basic science. The project-based intervention described in this study was designed to help connect cell biology knowledge with clinical practice, while developing students' interest in research-related skills by emphasizing experimental design with data analysis and problem-solving steps. Therefore, the discipline got closer to the students' professional activities, while progressively working on scientific communication skills, which may be neglected in the beginning of predoctoral dental education.

Continuous advances in materials, techniques, and technology in dental care create the need to train

professionals to become capable of identifying and dealing with those resources that are most effective in patient care and favor the use of scientifically proven materials and methods in their practices^{5,29} to provide their patients with the best possible treatment. Thus, the adequate understanding of basic sciences has a major role as a foundation for diagnostic reasoning.³⁰ In our study, students who participated in projectbased learning showed evidence of perceiving the applicability of basic sciences (represented by CB and in vitro assays) for the preclinical evaluation of dental materials. This notion was present in their reports in contrast to students who did not participate in the intervention (Figure 1, panel A). Most project-based learning students also indicated an understanding of important methodological aspects and issues related to conducting in vitro assays, including the choice for the best cell model and the use of controls and international standards (Figure 1, panel B and Table 3). Because the course focused largely on scientific methods, students learned concepts that would usually be accessible to them only as research lab trainees. According to the students' self-learning evaluation (Figure 4, panel D), the in vitro methods also contributed to their understanding of CB through a practical approach, making the course more interesting and challenging.

Because only 0.6% of Brazilian public high schools have specialized infrastructure including adequate science laboratories,31 the novelty of having a laboratory experience in the university could impact students' future choice for academic development after graduation.5 Laboratory activities with small groups of students are a well-known learning strategy, which has been found to be effective in promoting higher academic achievement.³² In our study, the initiative of most groups to work together to replicate a single experiment promoted a cooperative setting, preventing the discouragement of students from groups in which experimental errors could have produced misleading and low-quality results. This cooperative environment may also have positive effects on students' ability to solve problems, as discussed by Johnson.33

Considering that the aim of dental education is to prepare students to become competent dentists by developing a comprehensive understanding of a given clinical issue through adequate biological and medical concepts,¹¹ efforts should be taken to prepare these future professionals to seek out the best available scientific evidence, as in the principles of evidence-based dentistry.²⁹ However, the ability

to locate and identify high-quality evidence is still considered one of the main challenges in the transfer of knowledge from research to the clinical dental practice and decision making.³⁴ A previous study found that, while dental students usually rated their own literature research skills as good or excellent, many did not have the ability to perform advanced searches and assess the reliability of sources and differences between types of resources.35 Thus, our strategy approached the importance of such skills by exposing students to the systematic review of scientific databases such as Medline and PubMed, therefore presenting them with tools for an evidencebased practice,³⁶ of which most (85%) were unaware at the beginning of the course. Furthermore, the requirement for students to produce a viable experimental protocol and discuss the results on the final report possibly contributed to their greater involvement and care during the literature search. This effect was evidenced by most of the students' presenting adequate articles by the end of the database search and in the bibliographies of the reports, indicating a contextualization of the scientific literature during problem-solving in clinical practice.

Reasoning skills are often described as critical thinking and problem-solving capabilities, which are fundamental for improved clinical judgment, diagnosis, and decision making in the health professions.²¹ Therefore, problem-solving activities associated with research activities may promote the development of critical thinking, an important tool for the advance from novice to expert thinking in dentistry. The comparison of the final reports (Table 3) indicated that the project-based learning students were significantly more prone to define the purpose of the laboratory activities, restrict their claims to the supporting data, check inferences and consistencies of their claims by comparing their results with previous knowledge (from scientific literature) or interpretation of data (e.g., validation of controls), and consider the implications of their findings (e.g., by emphasizing their relevance for dental practice). Nevertheless, even though improvements provided by this intervention were previously discussed as important elements of reasoning and critical thinking,²⁸ further studies should be performed employing validated tools such as the California Critical Thinking Skills Test³⁷ to measure the impact of this learning strategy on the development of critical thinking.

According to Kanter and Konstantopoulos, courses based on research emphasize inquiry-based practices, engaging students in the evidence-based decision process, instead of memorizing static and theoretical facts in lecture-based courses.³⁸ Therefore, the positive results from our study encourage further investigations of this type of teaching strategy as an option for stimulating knowledge acquisition by problem-solving in a cooperative environment. Furthermore, the higher proportion of project-based learning students (78%, Figure 4, panel A) who recognized scientific research as a major contribution of CB to dental practice when compared to the other cohort (23%) reinforces the idea that laboratory activities can be effective alternatives as tools for the contextualization of research in dental curricula.^{39,40}

Since integrated curricula based on active learning are far from being a complete reality in dental schools worldwide, the teaching staff is often faced with the challenge of performing individual efforts at the discipline level to achieve, to some extent and under limitations, the goals of active learning in students' professional performance. Our study sought to determine if a single intervention, represented by a project-based course, could positively affect the learning of basic sciences.

One of the limitations of this study, from a research point of view, was the relatively small number of participating students and the study's inability to distinguish the combined effects of other courses from the curriculum. Nevertheless, the results, supported by the statistical analyses, indicate that both students' attitudes about CB and their acquisition of important scientific concepts were significantly affected by the single course intervention. Also, some evidence suggests that these effects might be longterm, as both the students' view of the CB role in dentistry and perceptions of relevant science-related concepts remained significantly different from the non-project-based-learning students four years after the intervention in their last semester of dental school.

It is important to note that this framework could be applied to other basic science disciplines, such as genetics, biochemistry, or physiology. It can also be adapted to diverse faculty backgrounds and availability of laboratory structure, as long as it has an adequate load of class hours and the organizers have research expertise on the selected theme. As an example, students could investigate the correlation of genetics and dental caries, employing a methodology based on literature search, presentation of seminars, design of protocols, data analysis, and production of final reports. Furthermore, in schools with a more integrated curriculum,^{5,11} interdisciplinary project-based initiatives might possibly connect diverse

scientific contents and even address the insufficient proportion of laboratory activities. With the involvement of more researchers and lab managers in the endeavor, there would be more student opportunities for lab visits and training. Nevertheless, more research should be conducted with larger sample sizes and other control groups that allow the application of adequate measuring tools and statistical models to further address the impact of these teaching and learning strategies, as well as their long-term effects, on the integration of basic sciences and clinical practice in dental education.

Conclusion

In this study, dental students in a projectbased course on cell biology demonstrated positive educational outcomes such as scientific knowledge acquisition and production of scientifically sound reports. These students also recognized the practical relevance of such knowledge to their professional development. These results suggest that a projectbased approach may help to contextualize scientific research in dental curricula.

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