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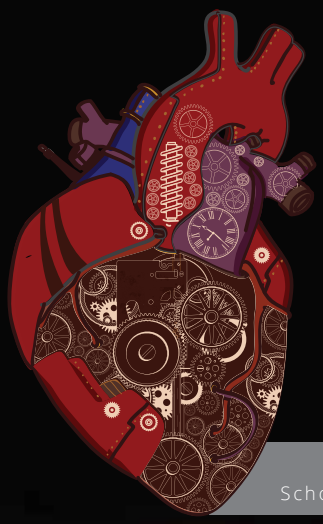
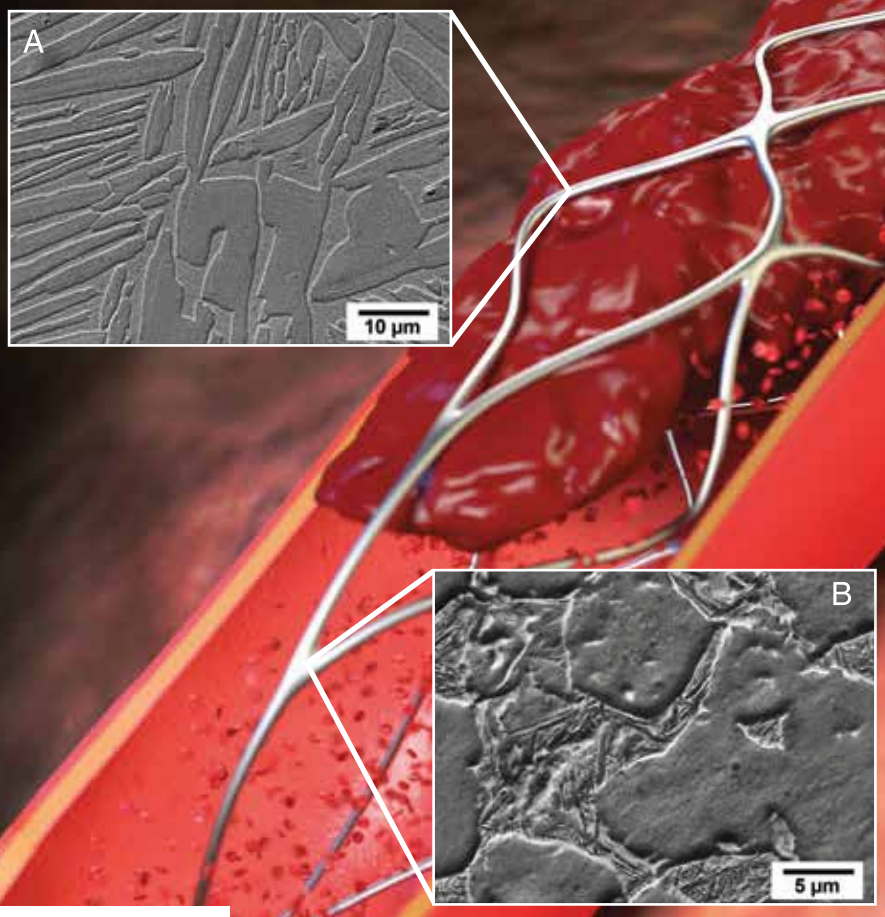


Journal of Engineering in Medical Devices

Official Journal of Medical Devices Engineering in Costa Rica
School of Materials Science and Engineering, Costa Rica Institute of Technology (TEC)



TEC | Tecnológico de Costa Rica

Credits

Journal of
Engineering
in Medical Devices

MIDM
Maestría en Ingeniería
en Dispositivos Médicos

This journal – the first of its kind in Costa Rica - showcases the work of students and faculty carried out as part of the focal point for scholarly basic and applied research on materials in the medical device field. Works carried out outside of the program on related topics are also welcome to submit a contribution.

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Main cover caption
Render of a vascular stent of a titanium alloy. Inset: scanning electron micrographs of Ti-6Al-7Nb; Image A: Quenched in liquid N_2 from 1010°C; Image B: Quenched in air from 960°C Heat treatments can change the microstructure of these kind of materials, which can significantly change their mechanical properties. Image A shows a typical martensite of alpha-Ti, while Image B shows a duplex alpha-Ti matrix with a lamellar alpha+beta structure within the equiaxed alpha grains. The latter is a stronger structure with good elongation properties *SEM images courtesy of Joaquín González, Costa Rica Institute of Technology.*

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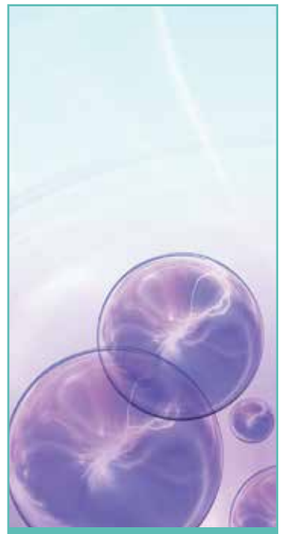

Welcome!

In the summer of 2013, CR government, industry and academic representatives led by CINDE traveled to the twin cities to visit with representatives of Minnesota's Medical Alley companies (e.g., Boston Scientific) and the University of Minnesota. The result was a memorandum of understanding between the CR Ministry of Science & Engineering and the U of MN College of Science & Engineering and a commitment to collaborate on educational programs to serve CR's medical device manufacturing industry. Faculty from the Tecnologico de Costa Rica (TEC) and U of MN's technological leadership institute (TLI) formed a partnership that helped shape TEC's Master in Medical Devices Engineering curriculum. TEC and TLI are bound by a common interest in preparing engineers and scientists to be technical leaders in a global medical device industry that reaches all the way from Maple Grove and Arden Hills to El Coyol, Global Park and Cartago.

Daniel L. (Dan) Mooradian, Ph.D.
Director of Graduate Studies
Medical Device Innovation at
University of Minnesota -
Technological Leadership
Institute

We would like to thank everyone who contributed to the development of the Master in Medical Devices Engineering program. We are particularly thankful to CINDE, the Costa Rican Economic Development Agency, for their advice and support. CINDE organized initial meetings and facilitated our interactions with the University of Minnesota and the visits to the Boston Scientific facilities to gain insight into the educational needs of the medical industry. We are also very thankful to our Costa Rica Institute of Technology President, Dr. Julio Calvo, for his trust and support and to the School of Materials Science and Engineering, for their support in the design and implementation of these Professional and Academic Programs. Along the way many companies also supported the program by providing scholarships to our students, providing classroom space, organizing manufacturing plant visits and also providing instructors whose expertise and knowledge have raised the quality of the program. To these companies, whose logos are presented in the last page, we are very thankful.

The first group of students - 29 students from the professional track and two students from the academic track - graduated from the program in March, 2017. The second group of students is underway and looking forward to present their graduation projects in October. The third group started in January 2017. The program is growing and we look forward to even greater interactions with the industry and with local hospitals and doctors.

The first edition of the Journal is an effort to present the work done by students in their graduation projects. A Technical Committee has been created to review submissions and ensure their quality. As we prepare for future editions we would like to invite you to submit original research for possible publication. In this way, you can share your knowledge with the broader Medical Device industry in Costa Rica and elsewhere, and others can gain from it.

Ricardo Esquivel I. Msc.
School of Materials Science and Engineering
Medical Devices Engineering Master Program
Coordinator Tecnológico de Costa Rica (TEC)

Journal of Engineering in Medical Devices

Tracing **the path**

It is with great pleasure that we publish this first issue of the Journal of Engineering in Medical Devices. The papers that are presented in this publication, collect selected works from the Graduate Program in Medical Devices Engineering of the Costa Rica Institute of Technology. As the Editorial Director, I am very excited to witness the outstanding progress and achievements from the program so far. Contributions from industry and academia that fit the scope of the journal are welcome to submit a paper. The Graduate Program, which is managed by the School of Materials Science and Engineering, is unique in the sense that both a Professional and an Academic Program are administered. In the Professional Program, students are usually engaged with the Medical Device Industry, as direct collaborators, entrepreneurs, consultants and independent contractors. In the Academic Program, students with an interest in carrying out high impact scientific research can work under the supervision of principal investigators with research projects in Universities, National Laboratories and Research Centers.

The contents of the journal are presented in three sections, which we hope will carry the essence of our program in each issue we publish here onwards. The first section, ***Applied Engineering***, presents contributions that apply the engineering design process to develop innovative medical devices, utilize problem solving methodology to the design of current medical devices, implement new designs and techniques for diagnosis and treatment, carry out design of experiments and statistical analyses, as well as modeling and simulation, to the optimization of processes in the development and manufacturing of medical devices. Contributions of graduation projects from students from the Professional Program will be included in this section.

The second section, ***Academic Focus***, summarizes the results of research projects from the Academic Program which have a high impact in a wide variety of areas of the research and development of medical devices, such as new materials for application in medical devices, design of innovative devices, new and improved manufacturing techniques, develop techniques for diagnosis and treatment, carry out design of experiments and statistical analyses, as well as advanced modeling and simulation of mechanical, thermal and functional behavior of medical devices.

The third section, ***Technical Notes***, present letters intended for fast diffusion of new projects, ideas, prototypes and developments from upcoming projects and research being carried in the Program.

Finally, I would like to thank all the contributors, supervisors and reviewers for their hard work in bringing this issue to a reality. Hopefully this is the start of a long journey.

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Journal of Engineering in Medical Devices

DEVELOPMENT OF A DETECTOR OF HEART MURMURS

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ABSTRACT

The most common cardiac afflictions are the heart murmurs, which are defined as abnormal noises during cardiac cycles. These abnormal noises are symptoms of a variety of cardiac pathologies, some of which can become very serious and put at risk the life of a person. Nowadays, heart murmurs can be precisely detected just by the combination of advanced equipment and a specialist physician. However, these resources are costly and scarce. Faced with this reality, is that the need for a low-cost and easy-to-use medical device that helps non-specialists improve their ability to detect and diagnose heart murmurs was identified. This investigation presents a prototype design proposal for a medical device, that would give capability to record, analyze and provide the first diagnosis of most common heart valve pathologies.

The design proposal was evaluated by a functional diagnostic test, using a simulator programed by computer. The results obtained show a sensitivity of 91.7% and a specificity of 82.6%, which are acceptable results compared to assessments made by other researchers of the topic.

It is concluded that the algorithm works properly during the initial assessment; however, it is required to perform additional evaluations with a larger sample of heart sound records, in order to achieve better statistical determination of configuration parameters.

Keywords: Heart, Murmur detector, diagnostic, device.

1. Introduction

The heart murmur is a condition that manifests itself as an abnormal noise in the cardiac activity, which is the result of a turbulent flow of blood through the heart valves [1]. In most cases, these abnormal noises do not pose a risk to the health of the patient [2]. However, in other case, they represent a serious danger to life and could lead to heart failure or blockage of arteries in the brain due to blood clots [3]. The diagnosing of these deficiencies of cardiac function is performed today by medical auscultation using a stethoscope, with a well-trained physician who can differentiate between normal and abnormal sounds of blood flow through the heart valves during the contraction and relaxation of the heart [4]. Nevertheless, this diagnosis is preliminary and usually if the result is positive for the existence of a murmur, it is required to continue with more complex studies to determine the anatomical failure, which have the disadvantage of being more complex and costly [2]. The need is highlighted here, then, to have a simple, practical, economical and effective way to detect such types of defects in the cardiac function, so that it is available to the population in a convenient manner at places of primary medical care [5].

In this research the design of a medical device for detection and diagnosis of heart murmurs is proposed by the registry (Phonocardiogram) and sound analysis of the cardiac activity [6].

To conduct such research, the current progress on the issue that researchers from several countries have carried out was reviewed. The most important results are shown, along with the methods by which the major technical challenges have been faced [5]. On the other hand, a systematic methodology followed to reach the final proposal for the design of the device was performed, counting with the help of eight staff physicians for the partial functional test of the algorithm.

Finally, the development and implementation of a computer simulator programmed in LabView to test, the functional algorithm is shown. Likewise, the results of the functional test performed with the medical staff are shown and the initial results of concordance, sensitivity and specificity of the device are discussed; as well as proposed conclusions and recommendations for future progress on the issue by other researchers.

2. Materials and Methods

Initially a research on the literature was conducted on reports that researchers in the field have published, and an assessment was done to establish the current offer in the market of related devices. Next, several proposals for a new design of a medical device for detection and diagnosis of heart murmurs were analyzed. The proposals were analyzed according to a table of positive and negative aspects of each. This methodology helped to choose the best proposal to be developed. Some of the aspects that had to be defined were: (1) dimensions of the device, (2) algorithm of classification and diagnosis of the cardiac sound (3) sound detection transducers and (4) external framework and interface.

After selecting the best proposal for the design of the medical device, an algorithm was designed using LabView™ (National Instruments) installed on a personal computer. With the programming in this language it was possible to evaluate some of the criteria for classification and diagnosis. This software was based on the analysis of the signal amplitude to provide diagnosis, unlike those created before, in which the most important characteristics of sound that have been analyzed were the intensity and frequency of their harmonicas.

The efficiency of the program was evaluated by analyzing heart sounds previously recorded and diagnosed, taken from the literature [7,8]. Sounds obtained were both from healthy people as well as patients with valvular dysfunction. The evaluation of the algorithm was performed using a statistical study of concordance by attributes.

For this study, the collaboration of 8 medical professionals was requested, who, together with the use of a computer with the algorithm for murmur detection, performed the diagnosis of 3 samples of phonocardiograms, with 3 repetitions each random sample. Neither the doctors nor the system programmed with the algorithm had access to the information about the confirmed diagnoses of the samples.

With this evaluation, the consistency and accuracy that a doctor may have to use the simulator for detection of murmurs, as support for an effective diagnosis was determined.
Selected audio samples have the following confirmed diagnoses, according to Table 1.

Table 1: Types of Phonocardiogram chosen to study efficiency of algorithm. Source: Authors

Phonocardiogram diagnosed
Normal Heart
Pulmonary Regurgitation
Regurgitation Tricuspid

To sum up, the outline of the study was that each evaluator conducted 9 diagnostic samples. The order in which the sound was presented to the evaluator to be diagnosed was randomized and the evaluator had no knowledge neither the order nor the true diagnosis of each case.

The concordance levels of diagnostics collected were evaluated by calculating the Kappa statistical index which represents the level of concordance of the classification of an evaluator compared to itself, with the reference value. The value of this indicator is between -1 and 1. Table 2 shows the interpretation of the indicator values.
In this research a Kappa value of 0.75 or more is considered as a good concordance value.

Besides the statistical index Kappa, sensitivity and specificity indicators were also calculated, which are widely used by researchers in the field, to determine the effectiveness of murmur diagnostic algorithms. This indicators were used in order to have a standardized measure of effectiveness that allows comparing the performance of the algorithm presented here with others developed previously.

Table 2. Interpretation of statistical Kappa. Source: Authors

Kappa statistic value	Interpretation
1	Total concordance
0	50% concordance
- 1	No concordance

These indicators are percentages and they are calculated as follows:

$$(1)\quad \text{Sensitivity} = \frac{tp}{(tp + fn)}$$

$$(2)\quad \text{Specificity} = \frac{tn}{(tn + fp)}$$

Source: Voss et al. 2005

Where *tp* is the number of true positives, *tn* the number of true negatives, *fp* the number of false positives and *fn* the number of false negatives.

A phonocardiogram will be classified as a murmur if it presents amplitudes of the signal above a comparison value in systole and diastole. In all cases, the simulation was able to detect the noises added to the systole and diastole, being able to detect the presence of a heart murmur. The comparison value chosen for systole and diastole was 0.35 of amplitude. The following report includes the full statistical analysis of the test of the efficiency of the algorithm as well as conclusions and recommendations of the research team for future advances in the field.

Results and Discussion

Development of the external physical configuration of the medical device detector of murmurs.

A brainstorming on possible configurations for the device was carried out resulting in the implementation of a central control unit connected by cables to at least three transducers that would turn the sound signal into an electrical signal. The electric transducers would be placed in the following sites of the patient chest where auscultation is commonly performed: aortic area, secondary aortic area, lung area, tricuspid area and the mitral area, choosing to place 3, 2 or 1 at the same time, depending on the technique and the type of murmur that needs to be identified.

Figure 1 shows the CAD design of the device, in the exterior form chosen. Note the transducers and the main control unit.

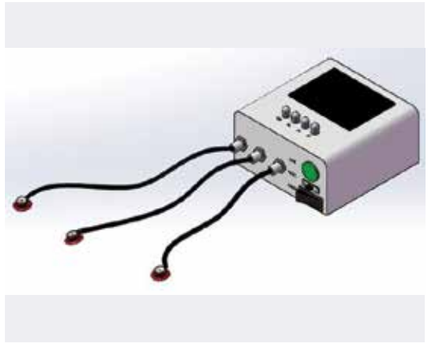

Figure 1 CAD Design of the heart murmur detector

Development of the murmur classification algorithm

Figure 2 shows graphically the typical appearance of a Phonocardiogram. In this graphic, the Y axis shows the sound amplitude while the X axis shows time.
Notice how at the beginning of each stage a high amplitude sound is located. These signals correspond to the characteristic sounds of the heart, which are easily audible with a stethoscope and happen to a more or less constant rate. The normal cardiac activity has 2 main sounds, called S1 and S2.

The first sound S1 is associated with the closure of the mitral and tricuspid valves. Both valves close almost simultaneously [1].
The second sound S2 is associated with the closure of the aortic and pulmonary valves. The aortic component precedes the lung, but generally cannot distinguish the closure of one over the other [1].

The location of each sound regarding the stages of the cardiac cycle is as follows: S1 is the main sound preceding the systole, S2 is the main sound preceding the diastole.

The proposal of this work to resolve heart murmur detection is to perform an identification of S1 and S2 by amplitude and time based on the information of the phonocardiogram, and also information about the time of the carotid pulse. The information of the phonocardiogram aids to locate S1 and S2 in the

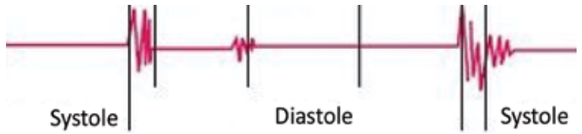

Figure 2. Section of a phonocardiogram showing the location of the systole and diastole. [1]

specific times, then not only the information of the phonocardiogram is used, but also the information of the times when the physician sees the carotid pulse in the patient, while recording the sound of cardiac activity in the phonocardiogram. Thus, the device will have two pieces of information for the analysis of S1 and S2. With the information corresponding to the temporal location of the carotid pulse, it will be possible to differentiate S1 of S2, because it is S1 that happens instants before the pulse is felt, so that the algorithm will temporarily associate S1 with the pulse.

It is important to note that different pathologies generate murmurs at different stages of the cardiac cycle. Then, if it is determined that the murmur is perceived during systole or diastole, this will add a differentiating factor between different pathologies. Table 3 shows a summary of the different pathologies that have been exemplified in this work and the corresponding location of the sound added in the cardiac cycle.

This information represents another feature to incorporate to the murmur detection algorithm according to the following statements:

A. *A sound recording with an added systole possibly corresponds to a heart with aortic / pulmonary stenosis or mitral or tricuspid regurgitation.*

B. *A sound recording with added diastole may correspond to a heart with aortic regurgitation or mitral or tricuspid stenosis.*

C. A sound recording with both a systole and diastole possibly corresponds to a heart with pulmonary regurgitation.

Table 3. Location of murmurs in cardiac cycle according to valvular disease. Source: Created based on information in [8].

Valvular pathology	Location of the murmur in the cardiac cycle
Aortic stenosis	Systole
Aortic regurgitation	Diastole
Pulmonary stenosis	Systole
Pulmonary regurgitation	Systole/ Diastole
Tricuspid stenosis	Diastole
Tricuspid regurgitation	Systole
Mitral stenosis	Diastole
Mitral regurgitation	Systole

In order to perform a limited test of the algorithm of identification with analysis of amplitude - time; a part of it is selected to be simulated by a computer.
The selected section helped identify three types of phonocardiograms: a normal heart, a heart with a murmur by tricuspid regurgitation and a heart with a murmur by pulmonary regurgitation.
Simulation, then, had a sample from a normal phonocardiogram, a sample from a heart with pulmonary regurgitation and a sample from a heart with tricuspid regurgitation.

All phonocardiograms were pre-recorded and pre-diagnosed according to sources of references [7,8]; all digitally in a .wav sound file format.

The sections of the algorithm related to the analysis of amplitude and time and the location of the

auscultation and the carotid pulse were not tested, because that information was not available in samples collected.

The partial simulation of the algorithm was performed with the use of LabView™. And the support of a programmer who was provided with all requirements that the program should meet, including the comparison algorithm of amplitude - time.

Figure 3 shows the interface to the user of the application of the developed simulation program for the murmur detection device. The LabView software was chosen as the right tool capable to partially implement the murmur detection algorithm for three out of the eight most common cardiac valve conditions. It has all the required capabilities to implement the rest of the algorithm and completely simulate the performance of the device. The user interface was well received by the physicians since it provided all the necessary information for a proper understanding and diagnosis of the phonocardiogram, such as the amount of cycles per minute, the systole and diastole segments and a heart animation that helps identify the phase of the cycle that is being analyzed.

Once the simulation concludes with the analysis of the Phonocardiogram, an output report is generated, an example as shown in Figure 4. The main output of the result is the diagnosis suggested by the simulator.

Through the evaluation it was possible to successfully test a section of the algorithm for the murmur detection, and the effectiveness of the following elements was demonstrated. Firstly, in a normal heart, S1 and S2 exceed an amplitude value that is not exceeded during systole and diastole: The value defined in the simulation was 0.3 - 0.4 of amplitude. The simulator was able to detect each S1 and S2 section of the phonocardiogram successfully. Secondly, S1 will have an interval length from 0.07 to 0.09 s and S2 will have an interval length from 0.08 to 0.1 s. In the simulation the chosen value was 0.08 seconds for both elements.

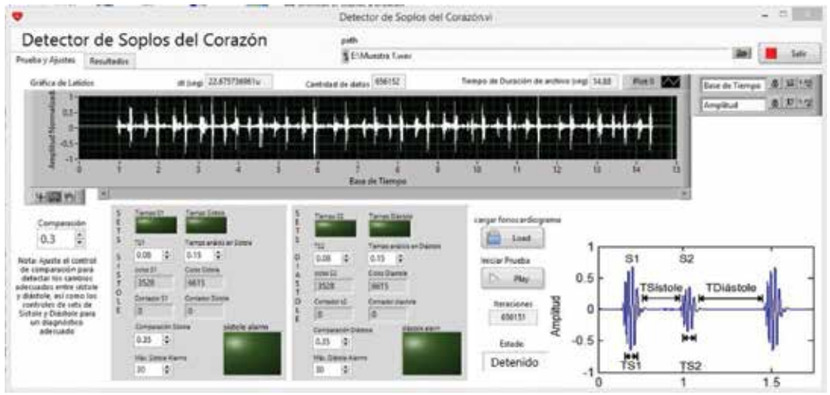

Figure 3. User interface for simulator of the murmur detector.

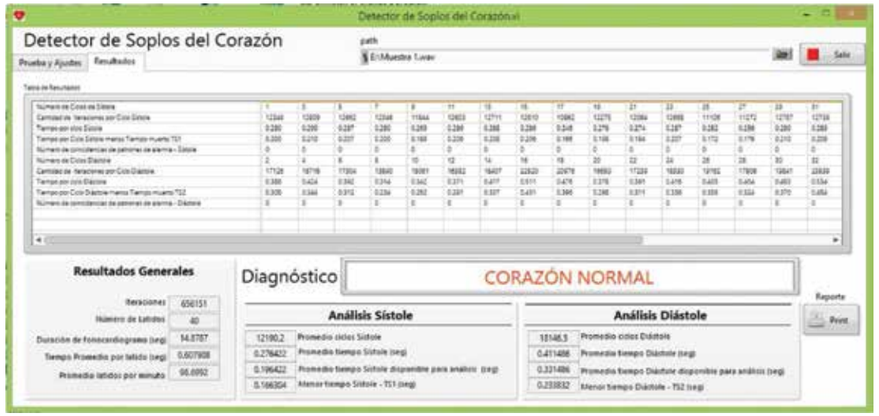

Figure 4. Results section of analysis from simulator of murmur detector.

Table 4. Summary of possible diagnoses scheduled in simulator.

Alarms	Diagnosis
No alarms	Normal heart
Alarms in systole	Tricuspid regurgitation
Alarms in systole	Pulmonary regurgitation

Table 5. Results obtained for number of hits, percentage of success and 95% confidence in the tests with the physicians. Statistical Kappa values are also shown.

Physician	Number of samples	Number of hits	Percentage of hits	95% CI	Statistical Kappas
1	9	7	77.78%	(39.99, 97.19)	0.66667
2	9	8	88.89%	(51.75, 99.72)	0.84071
3	9	5	55.56%	(21.20, 86.30)	0.36842
4	9	7	77.78%	(39.99, 97.19)	0.69492
5	9	9	100%	(71.69, 100.00)	1
6	9	8	88.89%	51.75, 99.72)	0.84071
7	9	7	77.78%	(39.99, 97.19)	0.68966
8	9	3	33.33	(7.49, 70.07)	0.11475

The simulator was able to correctly delimit S1 and S2, and not to confuse them with the components of systole and diastole, even if these were normal or with noise added. Thirdly, the time of systole is the shortest duration in the phonocardiogram of the S1 and systole set. During the test, it was found that the recommended values by the simulator for the time of analysis of the systole was 0.166 s in a normal heart, 0.622 s in a heart with pulmonary regurgitation and 0.267 in a heart with tricuspid regurgitation. In all cases, the values entered in the simulator were lower than these times. Fourthly, the time of diastole is the shortest duration in the phonocardiogram of the S2 and diastole set. The simulator recommended for the analysis time of diastole 0.234 s in a normal heart, 0.221 s in a heart with pulmonary regurgitation and 0.504 in a heart with tricuspid regurgitation. In all cases, the values entered in the simulator were lower than these times. Finally, all phonocardiograms that show amplitudes
above a value of comparison in systole and in diastole were classified as a murmur. In all cases, the simulation achieved to detect the noises added to the systole and diastole, being able to detect the presence of a cardiac murmur. The comparison value chosen for the systole and diastole was 0.35 of amplitude.
Once the simulator finishes analyzing the entire file of the phonogram, it uses the information of the recorded alarms to suggest a diagnosis. In this test, there have been three possible results, according to Table 4.

As noted, in each case, the diagnosis was correct. The simulator was able to perform the correct evaluation for the sample phonocardiograms selected. It was possible to verify that in each case, the simulator correctly detected sounds S1 and S2, extracting them from the rest of the cardiac cycle. Since most of public medical centers in Costa Rica are of primary assistance, usually general practitioners carry out diagnoses. Therefore, quick, low cost and easy-to-implement options are desired to aid the physician to deliver an accurate diagnosis.

The chosen statistical analysis was agreement by attributes. Table 5 shows the results obtained for the number of hits, the percentage of hits and the 95% confidence intervals for each of the participating physicians.

The results show that the level of success among the participating physicians is quite variable. The values obtained were from a 33% to a 100% success rate. These results, on average, give a level of success of 75%, which is an acceptable result. Because of the limited sample size, the ranges of the confidence intervals at 95% are quite large.

In addition, the Kappa statistic was calculated for each of the medical evaluators, in order to measure their success rate with respect to the true diagnosis of the samples used in the study. The results are also shown in Table 5. However, this exploratory study showed that agreement by attributes in this case was not acceptable. More studies with a larger sample size are required to support that this tool is a viable solution in primary care centers in Costa Rica, despite the good reviews from the participating physicians.

The calculation of the rates of sensitivity and specificity give a measurement of the effectiveness of the diagnosis made during the simulator's evaluation of the heart murmur detector device. The values for sensitivity and specificity calculated according to equations (1) and (2), respectively were 91.7% and 82.6%.
It is clear that for the physicians it is easy to identify that there is an abnormality in the blood flow, demonstrated by the good sensitivity result. However, in some cases it was difficult to classify each of the pathologies correctly, which shows that the device needs further improvement to include correct identification all the known conditions.

Based on the research, preparation of proposals and the analysis of the first results it was concluded that the implementation of an automatic device for detection and diagnosis of pathologies of the heart valves using phonocardiograms is possible, and the technology and knowledge needed are currently available to achieve this objective.

However, the objective of a device of murmurs detection is not to replace the skills of the medical staff in primary care center, but rather, to be of support for their daily work. A proper diagnosis of a cardiac pathology should not be limited to only one piece of information such as the automatic analysis of a phonocardiogram, but rather requires as many pieces of information about the patient, such as physical examination, medical history, auscultation examination, information about the symptoms the patient provides, among others. All this will help to improve the accuracy of diagnosis and the chances of recovery of the person.

Conclusions

1. The heart murmurs detector is a device that was accepted by the physicians during the study and it represents an opportunity for improvement of the primary care centers in Costa Rica.

2. It is evident that for the physicians it is easy to identify that there is an abnormality in the sound of blood flow, shown by the good result of sensitivity of 91.7%.

3. LabView programming software has all necessary capabilities to implement the rest of the algorithm and completely simulate the functioning of the device for the murmur detection.

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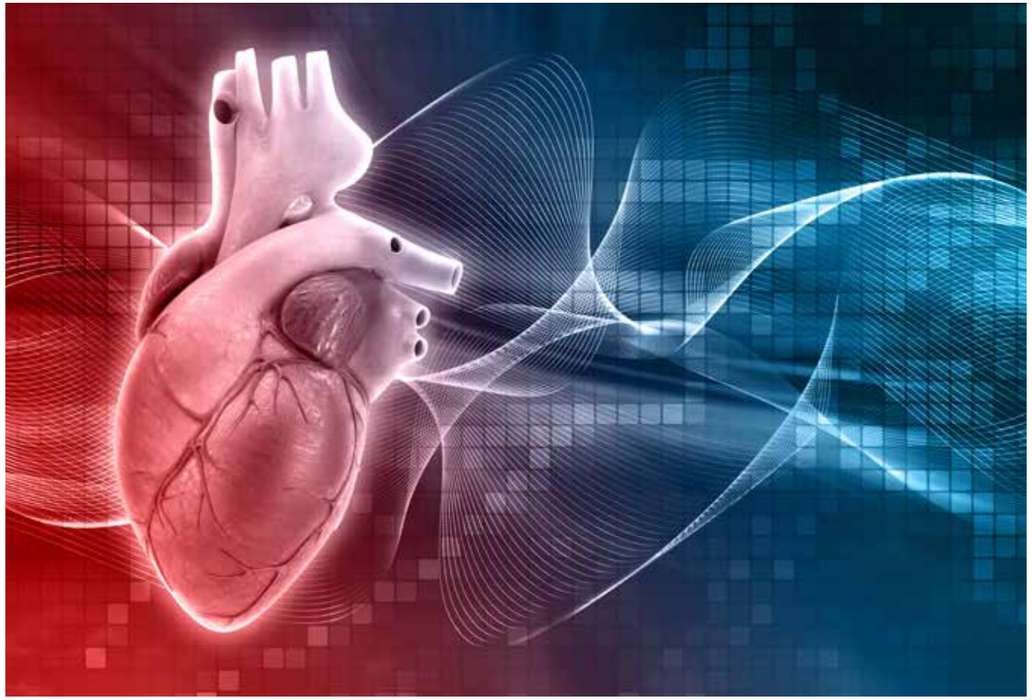

Promoting our program

Design of Medical Devices Conference

Participation in the 2017 Design of Medical Devices Conference, organized by the University of Minnesota, was a great success.

According to Ricardo Esquivel Isern, director of the Medical Devices Engineering Graduate Program at TEC, the participation with a stand in the conference exhibition fulfilled the main objective of strengthening ties and promoting our academic proposal.

Journal of Engineering in Medical Devices

EVALUATION AND IMPROVEMENT PROPOSAL FOR THE STERLIZATION PROCESS IN ONE OF THE HOSPITALS OF THE GREAT METROPOLITAN AREA (GMA) IN COSTA RICA

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ABSTRACT

The Sterilization Central of one of the major hospitals in the great metropolitan area, has significant opportunities for improvement, mainly in regards to the definition and measuring effectiveness and optimization of the sterilization processes used in this unit, as well as the process flow used to carry out its sterilization activities in the major instrument families and the operating room supplies (classified by the type and nature of the material they are made of).

The project shown in this work specifically intended to address these areas of improvement of the Sterilization Central, and collaborate with proposals for optimizing the process flow followed by the main families of products defined as “high demand” or “critical products” to be sterilized and which in turn allows a logical regulation with engineering and structured bases that leads to an optimal and standard process being safe for the patient, doctors, nurses and staff involved in the sterilization processes. In addition, a strategy for measuring the effectiveness of the sterilization process (mainly of these families, of input and instrumentation), currently used was proposed, in order to determine their effectiveness to achieve the purpose required of sterilizing, as well as the accomplishment of internal regulations of the Caja Costarricense del Seguro Social from Costa Rica as well as international regulations.

Keywords: sterilization, optimization, medical, devices, ethylene oxide.

Introduction

The activities carried out by a sterilization central in hospitals today play a transcendental role in relation to control of infections, which is a task of great professional relevance [1-4]. Hospitals and health facilities should provide quality health care to the population they serve and prevent further diseases arising from tenure in the health centre. One of these problems is nosocomial or hospital-acquired infection, which requires human and material resources for prevention [5]. Hospital infections are an important issue for their frequency, severity and economic impact, and are determined by the host, pathogens and environmental conditions in the hospital. While most of the infectious diseases in hospitals are endogenous, their frequencies are greater when there are a number of encouraging environmental circumstances [4,6].

The cleaning, disinfection and sterilization of medical devices are the primary and most effective elements to break the epidemiological chain of nosocomial infections. Sterilization stations contribute to the overall process of asepsis and antisepsis of the hospital [7]. The problems related to the structure and function of the sterilization centrals, are as complex as the structure of the hospitals. This complexity is justified by the important strategic position they occupy within the activity of the hospital. On the one hand they are aimed, as the rest of the hospital, to give adequate care for patients, but on the other hand they must be able to satisfy the needs and demands of the internal client, health workers of the center.

The disinfection-antisepsis and sterilization are procedures that are used to breach the chain of transmission of microorganisms, avoiding possible contamination of laboratories for the primary level of health care. Sterilization is the process by which the destruction of all forms of microbial life, including the spores takes place, so that the use of materials and equipment whose sterility is not guaranteed has serious consequences, such as increase in the rate of morbidity and mortality, economic impact, legal problems, as well as failures in the safety of nurse care [7]. Sterilization systems most used currently are steam, ethylene oxide gas and plasma using hydrogen peroxide [4].

The main function of a sterilization central is the decontamination of the septic elements and then to deliver them sterile. It is also advisable to carry out campaigns to train staff periodically to ensure efficiency of the service. Currently sterilization stations exist in two structures. One is total centralization, in which all phases of cleaning, disinfecting, preparation and packaging of the various materials and subsequent sterilization are executed. Partial centralization is acceptable where equipment suitable for prior cleaning and disinfection materials is missing, the staff is scarce or the physical plant is inappropriate [8].

It is important to highlight that the central of sterilization, as a producing center of the hospital, is subject to different regulations, which guarantee the safety of patients and the quality of health care, through the quality control of the procedures and the validation of processes, to ensure the safety and effectiveness of the sterilization process [7].

One of the main hospitals from the greater metropolitan area of Costa Rica has a department in charge of the sterilization process of all the instruments required in the operating rooms of this medical center. An example of such supplies ready for sterilization is shown in Figure 1. The sterilization process in this Hospital is of the total centralization type. Although there is a sterilization process and written protocols to carry it out depending on each type of input, it is advisable to make improvements on the process, infrastructure and security protocols with using ethylene oxide; based on the principles of quality and effectiveness on the sterilization processes.

Figure 1. Supplies of operating room on sterilization process.

To this effect, strong evidence of the reliability of such sterilization processes (regarding the use of chemical, physical and biological indicators) must be made in order that this will contribute solidly to the assessment and allow to have clear parameters regarding process that best ensure the sterilization required for major product families and instruments at the operating room. In addition, to determine if they have the equipment capacity to meet the requirements of reusable instruments and supplies sterilized in operating rooms (see Figure 2). In this work, the efficacy of the procedure for the sterilization was evaluated and improvements are proposed related to the distribution of the equipment and processes, as well as for safety measures that hopefully result in reducing of the risk of contracting nosocomial diseases at the hospital.

Figure 2. Equipment used at Sterilization Central of the Hospital.

Materials and Methodology

Through bibliographic analysis of existing national and international studies and visits to the hospital, there is a current diagnosis that works as a base for defining the problem and deepen into critical aspects of success as well as detailing in the types of indicators, different types of sterilization methods and technical aspects of the results that guarantee knowledge of the critical aspects and visualize the strategy to optimize the sterilization process. This involves aspects of distribution of the physical area and security measures that should be taken to ensure proper and safe sterilization processes according to the needs and current demand of the hospital center.

For the research, a comprehensive statistical analysis of exploratory nature was conducted, which was developed as follows:

Type of statistical operation:
Through analysis of the attributes whose chances of response are: Pass-Fail, qualification that depends on whether it meets with the specifications according to the results of microbiological tests performed to the medical instruments that are sterilized at the hospital. A binomial distribution B (np); where the population presents independent results and also do not vary from one test to another is used, hence probability of success (p) and failure (1-p) are constant.

Sampling Frame:
Random samples of hospital instruments of high demand needle holders, Adson forceps, fine scissors, dissections, nails removes, kidneys, separators, cups, straight halted, curved mosquito, speculums scalpel, channel probes, small forester and cables.These preliminary samples of exploratory character were taken in a stratified manner in 3 work shifts named as TA, TB and TC, for 3 different teams named Autoclave 2 (A2), Autoclave 3 (A3) and sterilizer of ethylene oxide (EO) which are represented in Table 1.

With respect to sample design it was developed with the same statistical formula shown [9].

Table 1. Nomenclature of samples, equipment and work shifts

Equipment	Sampling with respect to the execution of the sterilization process	Turn	Repetition
Autoclave 2:A2	before: a	TA	1
Autoclave 3:A3	after: d	TB	2
Ethylene oxide: EO		TC	3

Table 2. P and o q (1-p) values according to the team

Equipment	p values	Values of q (1-p)	Exploratory n values	Values of statistical n
Autoclave 2:A2	0.06	0.94	18	21*
Autoclave 3:A3	0	0	18	18
Ethylene oxide: EO	0	0	12	12

Based on the results of microbiological study p and q (1-p) value with a 95% confidence limit, are shown in Table 2 according to equipment.

The E value refers to the maximum value permissible in the estimation of p is defined theoretically as 10%.
In this way the values of n (sample size) are given in Table 2.

The objective of this study was to generate information on the current process, and to raise a proposal for improvement to the authorities of the hospital, through the validity provided by the statistical and experimentation procedures.

Microbiological tests were carried out in the Biotechnology Research Center of the Costa Rica Institute of Technology to the samples selected according to the sample sizes specified in Table 2. This information was very important to make decisions on the variation of the process flow to reduce the risk of contamination. However they are not shown in detail. Figure 3 and 4 show the images of sample microbiological cultures and the test tubes for analyses, which were carried out in triplicate.

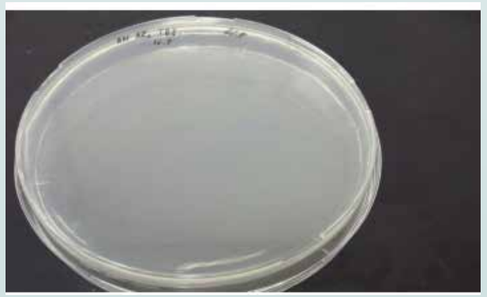

Figure 3. Sample microbiological cultures

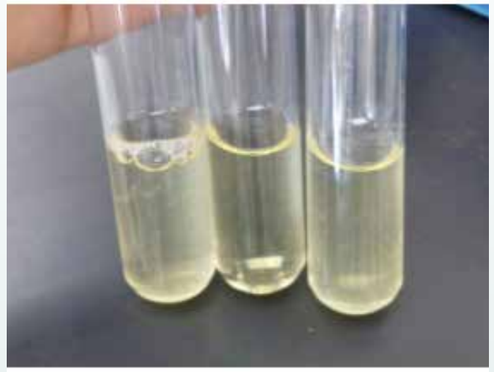

Figure 4. Analysis of results (in triplicate).

Through visits to the Sterilization Central from the Hospital, there was a documentation of the flow of process that the supplies coming from the operating rooms at the hospital undergo since they arrive as contaminated (materials, equipment, clothing and used sheets) and until they are shipped upon request as sterile equipment and material to be reused at the operating rooms.

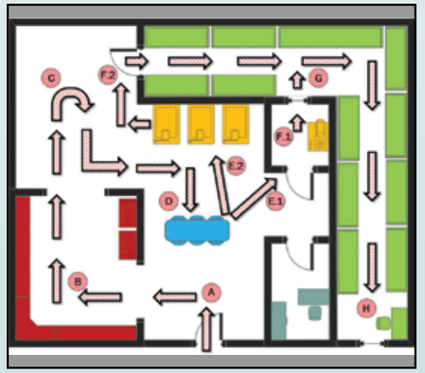

Figure 5. Current blueprint of sterilization

Results and Discussion

In Figure 5 a general layout of the sterilization central is shown, with the main stations and stages of the sterilization process indicated by letters, as well as the storage area and dispatching of sterile supplies to be re-used in the operating rooms.

Figure 5 also shows a description of the general process flow that these supplies follow within the central (depending on the the type of material they are made from, the different stages of the process are identified by different colors), and explains the steps and stages to be carried out until obtaining a supply currently considered by the central as sterile and suitable to be used again.

During the visits to the Sterilization Central, a very important specific need was identified related to treatment and use of ethylene oxide. The sterilization central lacks specific regulations based on engineering standards for handling this chemical during storage, transportation, use and disposal. Because of this, most of the staff working at this site as well as regular users entering the site are unaware of the health implications of the contact with this chemical. In addition, this lack of knowledge extends to even to the use and care of sterilization equipment, lack of real and validated methods for detecting possible gas leaks and the safety equipment that should be available at the site in case of an emergency.

In the hospital the main objective it is to preserve the safety of patients, nurses, doctors and users safety in general, while the procedures are successfully performed. Preventing nocosomial infections caused mainly by improper cleaning, disinfection and sterilization of surgical instrumentation, lies undoubtedly in suitable processes of sterilization in a proper and safe area, for which effective processes must be established according to the type of instrumentation and the requirements of disinfection.

This project had the main objective to provide a general and comprehensive option as an alternative to eradicate the risk of latent nocosomial infections in hospitals due to management of bio-waste and infectious material that can be controlled with the proper management of sterilization processes.

In this case, according to statistical research strategy presented in the previous section, (based on the samples of sterilization processes obtained from the hospital), it can be highlighted that the processes for washing, packaging and sterilization in the Hospital are efficient according to the culture studies.

According to the studies of the process flow in the sterilization central, a proposal is made to complement the current strategy of hospital work regarding the use of ethylene oxide as one of the means of sterilization. A need for improving the existing procedures a work program for the ethylene oxide is proposed to improve the safety of the users and staff of the hospital while enabling a better tracking and more efficient use of the chemical. Through a process of training the staff in charge of the use of the equipment can implement this step in the ethylene oxide sterilization unit.

Through a thorough analysis of the flow of the supplies carried out with the information collected in the field visits, a proposed flow for the material is presented . The least possible impact on infrastructure was attempted to minimize the potential costs of the change [10]. Movement of several equipment and the modification of the current work flow in order to integrate this structural modification. Figure 6 shows the results of such modification, which attempted to eliminate the issues highlighted in the current process flow, as detailed in the discussion of Figure 5. The changes are summarized as follows:

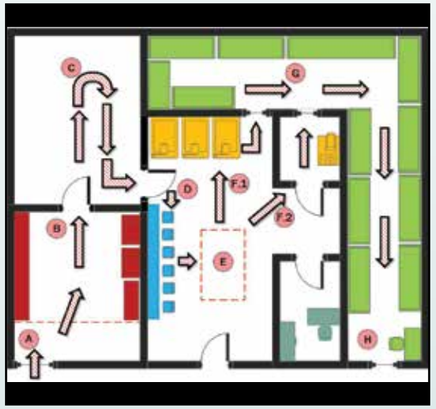

Figure 6. Sterilization Blueprint and Proposed Process

A: Independent entrance of contaminated material, which should be it is temporarily stored in this area behind the dotted line.
B: Washing area of contaminated material, divided according to the type of material that the supplies are made of (fabric, metal, plastic).

C: Storage area of washing material, isolated with doors to prevent cross-contamination.

D: Area of packaging of washed material prior to sterilization, larger area getting more workspace.

E: New temporary storage area of packaged material ready to be sterilized; achieving a buffer of material ready to be sterilized.

F.1: Autoclave sterilization for supplies made of fabric. A window or direct passage to the storage area is added from these autoclaves to avoid mixing sterilized supplies with the washed ones in Area C as it currently happens.

F.2: Isolated sterilization area by ethylene oxide for plastics and metal supplies.

G: Storage area of post sterilization materials.

H: Dispatching area upon request of sterilized supplies ready for use in operating rooms.

The results expected from the implementation of these structural changes and process flow are a decreased risk of infection and spread of diseases by allowing an independent entrance of the contaminated material to the central, which is one of the main achievements of this study. Also, a decrease of the overall cycle time of the process and decrease of possible failures by diminishing the amount of movements, which increases the efficiency and reduces costs [9-11]. The new process allows better management and protection of the unsterilized material by implementing two new temporary storage areas (storage area of contaminated material in Figure 6, point A) and a storage area of packaged material prior to sterilization , see Figure 6 - Point E). With the addition of the two temporary storage areas mentioned above, there is an improvement in the efficiency on the total cycle time of the central by being

able to allow the entry of more contaminated material while obtaining two buffers of material on current bottlenecks of the process, such as the space for the entry of contaminated material and the space for the material prior to sterilization.

The new design aims to separate the entrances to the storage area post sterilization, this is especially important for the output of sterile material that comes from the Autoclaves since it should not be mixed in the area with the contaminated product that has only been washed (unsterilized), as is currently the case.

In general terms, with this relocation and accommodation of systems and furniture in the Central, the workspace is increased in regards to the current design, such as expansion of the packing table, as shown in Figure 6, Point D.

Conclusions

It can be confirmed that sterilization processes can be effective with regards to eliminating microorganisms in instruments and surgical clothing in the sterilization central of the Hospital where the study was performed. Inadequate practices related to the workflow process, transfer of material and use of chemicals, can increase the risk of nocosomial infections to personnel working in the hospital and the patients. For this reason, effective processes to eliminate areas of contaminating micro-organisms, proper process flows, and effective management of chemicals are vital to run a sterilization central safely.

In order to obtain a higher degree of confidence and efficiency in the sterilization process, the following recommendations are proposed:

1. The flow of current sterilization process was analyzed and multiple points of improvement were identified both in the process, as in the accommodation of supplies (redistribution of the internal space) and infrastructure; which it led to generate a proposal to redesign the process flow within the sterilization central, as well as a structural modification as minimally invasive as possible that would go hand in hand with the redesign of the process flow to achieve an improvement in the efficacy and safety of the sterilization process. The proposal for a redistribution of the physical area is a proposal that is generated when visualizing within the general framework the need to guide process flows of the hospital and take the opportunities for improvement in this regard to ensure a logical and orderly process that allows a flow of a more efficient and safe process.

2. A work program with ethylene oxide to specify safety practices is proposed, to clarify the types of environmental and individual monitoring for the use of ethylene oxide in addition to defining environmental controls to mitigate potential environmental impacts with the expectation that this proposal is a reality that places the hospital at the forefront of the proper treatment when ethylene oxide is used as a sterilization method regarding the other hospitals in Costa Rica.

Acknowledgements

The authors thank the Biotechnology Research Center of the Costa Rica Institute of Technology for their support in conducting the microbiological tests. Also the authors thank Dr. Francini González Rojas, Head of the Central of Sterilization of the hospital where the research project was developed, for her vital consent and collaboration to the success of this work.

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Journal of Engineering in Medical Devices

OPTIMAL POSITIONING FOR ACETABULAR CUP AND CANCELLOUS FIXATION SCREWS IN TOTAL HIP REPLACEMENT SURGERY

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ABSTRACT

Acetabular cup positioning in total hip arthroplasty has become the most problematic part in this type of orthopedic surgery, given that there are a series of factors which are difficult to control, such as age, gender, medical conditions, reduced space and approach chosen. In this article, twelve cadaveric pelvises were analyzed by image processing and CAD software, to find the optimal combinations between the fundamental angles involved in cup orientation. The results of this work show that cup rotation between the first and third quadrants of safe zones could withhold longer screws without putting at risk important structures surrounding the pelvis. Optimal anteversion and abduction angles also help to avoid danger areas and a more serious complication such as dislocation in a hip replacement. The analyses show that 30 - 40 mm screws fit without risk with 15° ± 5° anteversion and 45° ± 5° abduction angles, if they are in the first and third quadrants of the pelvis. Any screw outside this area could seriously risk one or more structures near the pelvis, with dangerous consequences to the patient.

Keywords: Acetabular cup, total hip replacement, screw positioning, safe zones

Introduction

Acetabular cup positioning has a significant effect on the outcome of total hip arthroplasty (THA) and often leads to dislocation altogether with factors like age, sex, medical conditions and the surgical approach selected [1]. The surgeon though, has control over the surgical approach, the implant selected and mainly the implant positioning. However, implant positioning is often a difficult variable to control during minimal invasive surgery given that the intraoperative surgical space is reduced and visibility is limited. To describe a method that quantified and averaged data gathered in human specimens Krebs et al. [2] developed a standard representation of the pelvis, where topographic features such as the ilium and ischium allowed a reconstruction of the hip. They divided the pelvis in four zones and used these areas to define the safe zones of the acetabulum, such as the superior iliac column and the area between the angle of the top sciatic notch and the line that bisects the ischium.

Wasielewski et al. [3] found four quadrant zones reliable for screw placement, drawing a line dividing the pelvis by the ASIS (Anterior Superior Iliac Spine) to the posterior fovea and a second line perpendicular at the midpoint of the acetabulum. The study found that the anterior quadrants, superior or inferior, should be avoided given that structures like the external iliac and obturator nerves, arteries and veins are highly at risk, in contrast with posterior superior and inferior quadrants, which contain in most cases enough available bone stock for proper attachment of the screws.

Sotereanos et al. [4] provided three bony landmarks, identified intraoperatively as ilium, superior pubic ramus and superior acetabulum, to define a plane, and based on it determine a suitable cup anteversion or retroversion. The plane can provide a relationship between the acetabulum and the structures at risk behind it. However, there is no established relationship between this plane and the safe zones at moment. The planes used to define abduction and anteversion, in relation with conventional CT scans, were defined in the study by Stem et al. [5], where 100 CT scans were analyzed to accurately outline a normal range of acetabular abduction angles as 31° - 46° and anteversion angle of 23° [6].

A wide range of abduction (40° ± 10°) and anteversion angles (15° ± 10°) are used to lower the probability of hip dislocation and to make sure a good bone stock area is available [7]. Liu & Gross [8] also confirmed that in abduction angles over 50° failures were not observed.

Even though the safe zones and proper angles of anteversion and retroversion are a good guide during a surgical procedure, awareness of where the screws will fit without interfering with cortical zones is crucial to avoid injuries in vital intrapelvic structures and key for an optimal acetabular cup positioning [9].

The current study is intended to validate the safe zones exposed by Wasielewski et al. [3], based on three-dimensional CAD models, as well as to determine the position of the cup that accepts the longest length of screws conforming with good bone stock. Also, it is intended to study the angle in which the screw has the optimal amount of bone stock available to improve fixation and lower the risk of injury.

2 Materials and methods

A total of 12 cadaveric pelvises were scanned by Computer Tomography (CT). These specimens were divided in 6 Japanese and 6 Caucasian cases (3 men/ 3 women for both). A Design of Experiments Analysis (DOE) using a full factorial design with 4 factors was applied. The factors were the acetabular anteversion angle, the abduction angle, the screw hole utilized for a specific model of an acetabular cup and the patients' gender.

The cup size was determined by Argenson et al. [10], who used the CT view passing through the center of the acetabulum, which determines preoperatively the size that would match the anatomy of the patient. The acetabular anteversion angle was considered the angle measured from a transverse view starting from the sagittal plane up to the line that bisects the femoral head and femoral neck. This angle is recommended to be 15° ± 10°. The acetabular abduction angle was considered a measurement calculated from the horizontal line formed by the triradiate cartilages and the line parallel to the surface of the acetabular cup. This angle is recommended to be 40° ± 10° [4].

The image segmentation operation was performed with MIMICSTM software version 16. Furthermore, the images were converted into IGES (Initial Graphics Exchange Specification) models in 3-Matic 8.0 preparing them to then import into SolidWorksTM 2013 to perform the DOE experiment. To be modeled in MIMICS, the CT images were required to have at least 10 mm proximal from the ASIS, the acetabulum, the acetabular fossa and the pelvis symphysis which is the soft tissue between the pubic tubercles. This process is shown schematically in Figure 1.

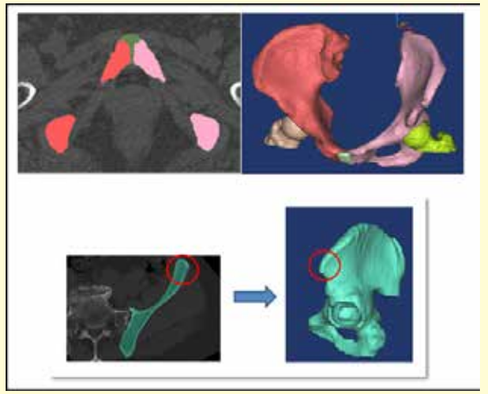

Figure 1 Image segmentation of the study

The response variables associated with the study were the optimal angle with enough available bone stock, the maximum screw length, and the distance to the cortical bone for the corresponding maximum screw length. The maximum screw length response variable corresponds to the maximum size that fits before intersecting with the cortical bone. The optimal screw angle was measured as the angle that holds more bone stock and was not likely to intersect or trespass the cortical bone. The cup orientation was defined by the anteversion and abduction angles. A parallel plane to the surface of the cup was created to set the rotation that will then determine the safe zones. Every cup rotation was analyzed within all the possible combination of angles and genres. The DOE response variables are shown schematically in Figure 2.

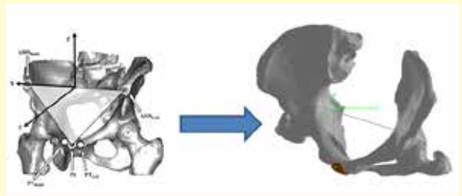

Figure 2 DOE experiment variables graphical description

3 Results and discussion

3.1 Maximum screw length allowed

Some of the data was analyzed manually so that critical points could be determined before analyzing Minitab results. From the obtained data, the maximum screw length was defined depending on the anteversion, abduction and rotation angles. Figure 6 shows the averaged maximum screw length according to the rotation defined. As the rotation increases above 45° the allowable screw length is shorter, which can be explained given that the morphology of the hip bone on that rotation angle is narrower than for the rest of the rotation angles. On the other hand, above 113° the screw length remains constant.

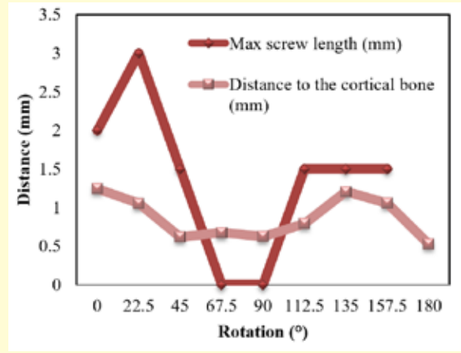

Figure 3. Averaged maximum screw length per rotation

As shown in Figure 4, for a 22.5° rotation, a longer screw is allowed with an abduction angle of 50° when compared with 30°. It is possible that a longer screw can be used if the cup is oriented with an anteversion of 25°. The screw length behavior for a 67.5° rotation is very similar to 90°, where a high value anteversion does not allow screws longer than 2 cm, while 50° abduction does. On the other hand, the longer screw allowed in a pelvis

is 30 mm long, any longer screw could represent a high risk for the surrounding nerves and structures of the pelvis.

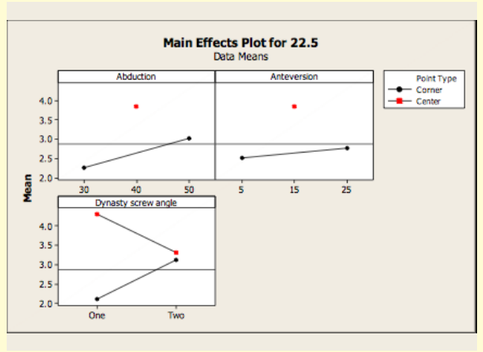

Figure 4 Main effects plot for a 22.5° rotation

As far as interactions between factors is concerned, for maximum length at 22.5°, Figure 5 shows the high anteversion and abduction angles represent a better screw length result. The gender factor does not generate any interaction however; Dynasty screw angle one altogether with abduction of 40° and anteversion of 15°, maximizes the allowable screw length.

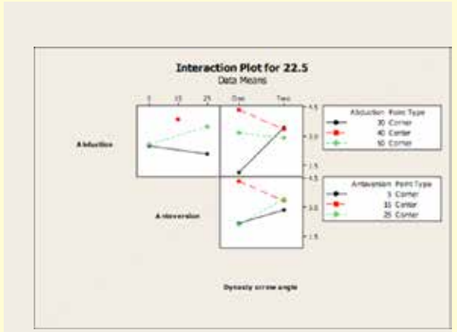

Figure 5 Interaction plot for 22.5°

3.2 Minimum distance to the cortical bone

The overall results of the distance to the cortical bone are shown in Figure 6. The small angulations represent better options of space available between the screw and the pelvis. This also happens with the 112.5° and 157.5° cup rotations; in these sets of rotations the risk of interfering with surrounding structures is considerably lowered. With more space available between the screw and the cortical bone, there is a lower chance of the screw running through the pelvis and causing a more serious injury in the surrounding structures.

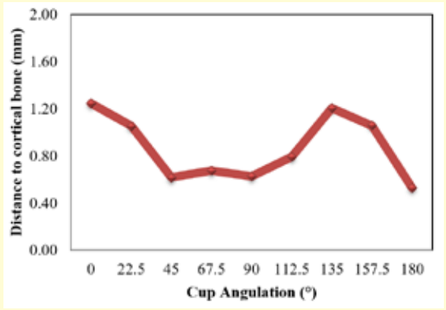

Figure 6 Average distance to the cortical bone per rotation angle

It is also possible to relate these results with those obtained in the maximum screw length allowed in the pelvis, given that with a 22.5° rotation, a 30 mm screw fits with considerable space surrounding it, which would mean a safe path in surgery. These results can be seen in Figure 7. If the results are seen by rotation angle, it can be noted that in an abduction angle of 50° the distance to the cortical bone is averagely higher than in the 30° angle. The previously discussed result justifies the facts that the majority of surgeons utilize a 40° abduction angle for the acetabular cup, since it gives good outcomes.

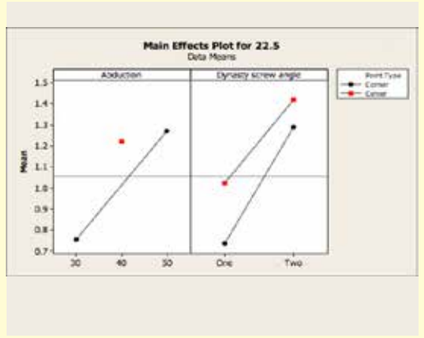

Figure 7. Main effect plot for 22.5°

A survey of the equipment of computerized tomogIn the interactions between independent variables, the distance to the cortical bone is lowered with a maximum abduction and anteversion, as shown by Figure 8. In most of the rotation angles the interactions are significant ($p<0.005$), in a tertiary level. However, the ones that show a considerable change are those that utilize optimal abduction and anteversion angles together with Dynasty screw angle two, in both female and male genders. Based on the study it can be determined that the results obtained when obtuse angles are used, guarantee longer screw lengths and safe distances to the cortical bone.

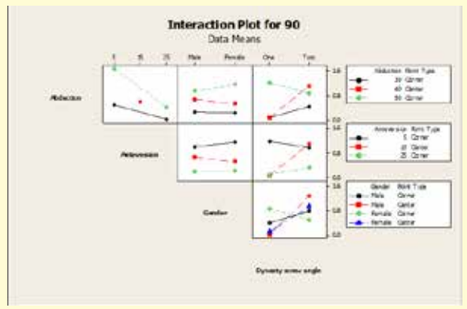

Figure 8 Interaction plot for 90°

3.3 Evaluation of optimal screw angle

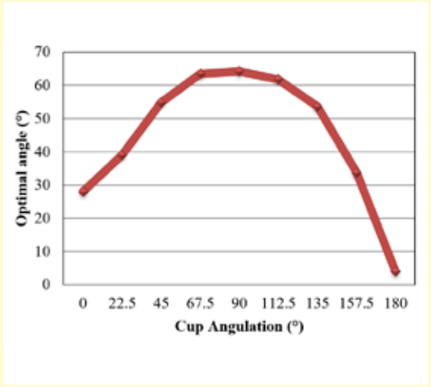

Figure 9 Averaged optimal screw angle for each rotation angle

Figure 9 shows that the screws adapt themselves to the anatomy in the pelvis as the rotation angle increases. In general terms, without taking into account the angles from the limit values (0° and 180°) the optimal screw angle values are similar in the following combinations: 22.5° with 157.5°, 45° with 135° and 67.5° along with 90° and 112.5°.

The optimal screw angle is the response variable that is most affected by gender, this because male and female pelvis anatomies are different. Examples of the main effects obtained in the design of experiments performed are shown in Figure 10. If a future cup design is required, the interaction between these angulations should be taken into account, given that this experiment portraits different types of anatomies and they all behave averagely as stated before.

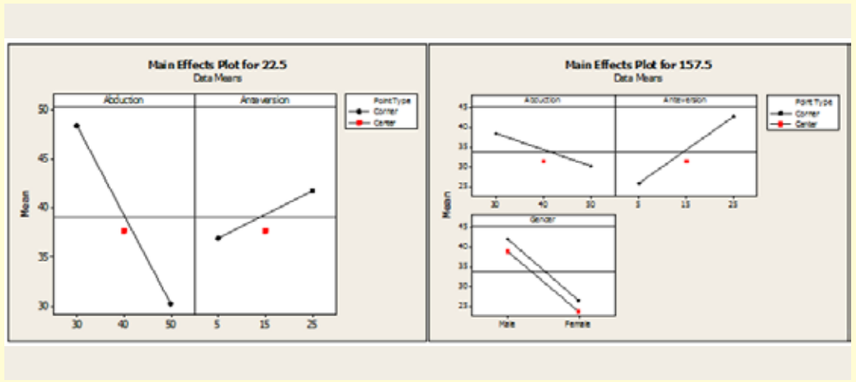

Figure 10 Main effect plots for 157.5°

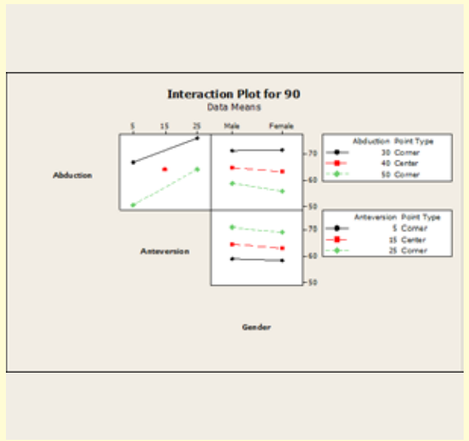

Figure 11 Interaction plot for 90°

In the analysis of the interactions the rotation angles with statistical significance, with any abduction angle and high values of anteversion (25°), the optimal angle increases. Regarding the gender differences, the main effects for each rotation were analyzed using an angle of abduction of 30° and maximum anteversion angle. The optimal screw angle increases for the majority of cases except for a rotation of 45° where female screw angle increases but male screw angle decreases instead. This means that in the rotation angles of 90°, 112.5°, 135°, 157.5° and 180° males would require a higher value for the screw angle.

Ji et al. [11] supported differences between gender however, the role of the gender factor is statistically significant for the optimal screw angle response variable. The viability of having cups designed specifically for either man or woman needs to be analyzed in terms of costs and manufacturing.

4. Conclusions

This study was based on the Dynasty® acetabular cup of Microport Orthopedics and because of this, there is no previous data based on 3D studies for this specific acetabular cup. Nevertheless some of the collected data can be used for future optimization of acetabular cups designs in the medical devices field.

The resulting data showed an opportunity of improving the angulation of the screw holes in the acetabular cup, to avoid risk structures as the sciatic nerve and also, prevent further dislocation or pain caused by a misguidedly positioned acetabular cup. The obtained data determined the optimal rotation to be used after abduction and anteversion angles have been chosen by the surgeon. The optimal abduction angle is between 30° - 40° and anteversion angles between 15° - 25° have proven to have better outcomes according to the results. However, rotation must always be kept between 0° - 45° or 112.5° - 157.5° from the Anterior Superior Iliac Spine, otherwise the statistical results show a higher chance of pelvis puncture.

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Journal of Engineering in Medical Devices

ADVANCING IN THE QUEST FOR SMARTER IMPLANTS:

A BIOACTIVE AND ANTIBACTERIAL PLASMA SPRAYED COATING ON BIOCOMPATIBLE POLYMERS

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ABSTRACT

The use of polymers in the field of orthopedics and tissue engineering is leading to new frontiers in its applications. Usually, it is desired for implants to support bone in-growth and to enhance osseointegration as well as to avoid post-surgical complications like bacterial infections. When these requirements are met, new advances in the quest for smarter implants can be achieved. Being Atmospheric Plasma Spray (APS) the most widely used technology for surfacing metal implants, the coating of polymers is a step towards the goal for smarter implants. Nonetheless, plasma sprayed coatings have not been widely studied on polymers. Therefore, the aim of this study was to plasma spray polymer samples with bioactive and antibacterial materials, in order prove their viability, and analyze their micro-structure and stability, as well as determine their equivalence with plasma sprayed coatings over metals. Characterization of samples was performed using visual inspection and scanning electron microscopy. Results indicate comparable outcomes with plasma sprayed coatings over metals, proving the viability for bioactive and antibacterial plasma sprayed coatings over polymers. Also, bone cells adhesion to the treated samples show the clinical potential in the quest for smarter biomaterials.

1. Introduction

The use of polymers in modern medicine plays an important role. Advantages like their low specific weight, cost, and performance, has led to new frontiers regarding applications and patient customization in the field of orthopedics and tissue engineering [1,2]. Usually, it is desired for implants to support bone in-growth and to enhance osseointegration with the remaining bone or tissue structure, as well as to avoid post-surgical complications like bacterial infections [3]. When the combination of material, bioactivity and antibacterial properties is met, new advances in the quest for smarter implants can be achieved. To accomplish this purpose, these medical devices can be coated with different materials through technologies like thermal spray. Among different thermal spray techniques, Atmospheric Plasma Spray (APS) is the most widely used technology for surfacing implants [3-7]. Nevertheless, plasma sprayed coatings have been primarily applied to metallic substrates, and unfortunately, only a few studies are available on polymers and composite materials [8]. Therefore, the aim of this study was to coat polymeric samples with bioactive and antibacterial materials. Polymeric substrates chosen were poly-ether-ether-ketone (PEEK), polylactic acid (PLA) and polyvinyl alcohol (PVA), which are commonly used in orthopedics and tissue engineering applications [1,9,10].

The coating materials used in this work were selected in the one hand to promote bioactivity in the surface of the substrate, and on the other hand, to avoid bacterial propagation at a lower cost than silver ions and nanoparticles [11]. Regarding bioactive materials, hydroxyapatite (HAp, $[Ca_5(PO_4)_3OH]$) is a widely-known mineral that promotes osseointegration [12], and titanium dioxide is often sprayed on titanium alloy implants to build apatite similar to the bone in the surface, improving the adhesion force between the implant and the bone [5]. Furthermore, titanium dioxide (TiO_2) also has also been reported to have antibacterial properties [11]. Copper (Cu) was also used as antibacterial material for the experiments, which along with zinc, hold a promise as potent antibacterial agents on a wide spectrum of bacterial species [11].

Due to the physics and experimental nature of the APS process, and the few references about plasma sprayed coatings over polymers, the experimentation was conducted first based on an empirical basis to determine adequate parameters to obtain appropriate coatings. In this case, coatings were evaluated in terms of substrate shape (non-deformation of the polymeric probes), stability and morphology of the coating (macro and micro analysis); using simple visual characterization (20 cm from sight) and scanning electron microscopy (SEM) imaging. The main goal of the study was to obtain depositions over polymers that are equivalent to depositions over metals, mostly in the terms of their microstructure.

2.Materials and methods

2.1. Test materials

High purity biocompatible hydroxyapatite (Oerlikon MetcoTM 6902 Hydroxylapatite) was used, with particle size between 50 and 160 µm, and at least 95% calcium phosphate.

The titanium dioxide used (Sulzer MetcoTM AmdryTM 6505) had a chemical composition of (wt. %) 99.0 TiO2, <0.2 TiO2, <0.2 Fe2O3, <0.2 SiO2, and <0.5 of other elements. Particle size was reported by vendor as 45 +/- 5 µm.
For the antibacterial coatings, a mix of 97 wt.% hydroxyapatite (MetcoTM 6902) and 3 wt.% copper

MetcoTM 55 was used. The powder was treated using a mixer for two hours and dried at 60 °C for 12 hours. The copper used is classified as pure metal, copper base powder, with chemical composition of 99.0+ wt.% Cu, and <0.7 (maximum) of other elements. The nominal particle size distribution reported by the vendor was 90+/- 38 µm.

The substrate materials were 11 mm diameter discs of PEEK, PLA and PVA. The PEEK was cut from a 1 mm thickness sheet of semi-crystalline condition. PLA and PVA discs were obtained by a 3D printer, from a EasyFilTM PLA and AquaSolveTM PVA spools, respectively, both in natural color.

2.2. Equipment used and experiment setup

The plasma spray equipment used –located at the Leibniz Institute for Plasma Science and Technology (INP Greifswald), Germany– was Oerlikon Metco™ System Platform MultiCoat™, with one F4MB-XL plasma torch. For the HAp and HAp+3 wt.% Cu coatings, plasmatron power was set at around 24 kW, while for the TiO_2 coatings, power was set near 45 kW.

Substrates were placed at different stand-off distances (distance from the exit of the plasma torch to the surface to be coated) to evaluate their shape after exposure to the high temperature plasma, as well as the coating apparent adherence to the substrate. This is, the formation of a closed layer (substrate completely covered) or an open layer (substrate can be seen). Also, the quantity of layers and the cooling time between each coating, were characteristics taken into consideration.

Additionally, the influence of the roughness of the substrate was tested. Corundum blasting (tiny alumina pellets) was applied to some substrates, while others maintained their natural finish. To test coating stability, the probes were place in ultrasonic bath with ultrapure water, for 15 minutes.

The microstructure, chemical composition and wettability of the coating are key factors for a proper adhesion of the bone tissue in the surface of implants. To test the bioactivity of the coatings, bone cells (osteoblasts MG63) were cultured over the PEEK substrates coated with TiO_2+HAp, for one hour on a 2.5% SBF serum and 50,000 cells/600 microliters.

2.3. Characterization methods

Visual characterization with no magnification, at 20 cm from the eye was performed to evaluate coating macrostructure (closed or open layer), and changes in color and shape of the of the substrate. For splats microstructure, coating stability and adhesion evaluation of MG63 osteoblasts, a Hitachi TM-1000 scanning electron microscope (SEM), with acceleration voltage of 15 kV, was used. For roughness characterization, profilometer Zeta-20 from Zeta Instruments, was used.

3. Results and discussion

Overall, the best deposition results were obtained for the PEEK substrates, due to its higher melting point compared to PLA and PVA, making it easier to deposit several layers in order to accomplish a high-quality coating. Also, there was no appreciable deformation or temperature damage of the substrate itself.

Regardless of the stand-off distance used and even with only one layer deposited, PLA and PVA substrates showed some degree of deformation after exposure to the plasma spray, as well as some burning marks and blisters, as shown in Figure 1. Figure 1(a) shows the case of the PVA substrates, whereas Figure 1(b) the PLA substrates. The latter were more significantly damaged by the temperature of the plasma spray. Therefore, the focus of

Fig. 1. Deformation presented in samples after exposed to high temperature plasma: (a) PVA substrates, (b) PLA substrates

the study from here onwards relies on depositions over PEEK substrates.

One of the most important results obtained, is that when applying a TiO_2 layer prior to the application of HAp, the former creates a good bonding layer for the HAp to adhere to the substrate. The outcome, as shown in Figure 2, is a closed deposited layer, where the substrate surface totally covered, giving better results than when applying even more layers exclusively of HAp. Figure 2(a) shows the case of a deposition of 10 passes of HAp with no prior TiO_2 deposition. An open area in the deposition in the upper left corner can be observed as a darker color. Figure 2(b) shows the case of a deposition of 2 passes of TiO_2 prior to 6 passes of HAp, and Figure 2(c) of 2 passes of TiO_2 prior to 4 passes of HAp. In both cases a closed deposition was achieved.

Also, the effect of substrate preparation on adherence properties, roughness in this case, was evaluated. Figure 3 shows samples sprayed under the same conditions, but with different surface roughness: column (a) corresponds to PEEK substrates without any treatment (“Polish”) and an average roughness R_a=1,86 µm, while column (b) displays substrates treated with corundum blasting (“Rau”) and average roughness R_a=4,29 µm. It is clear how all the samples shown by Figure 3(a) retain less coating material than samples with rougher surface, as

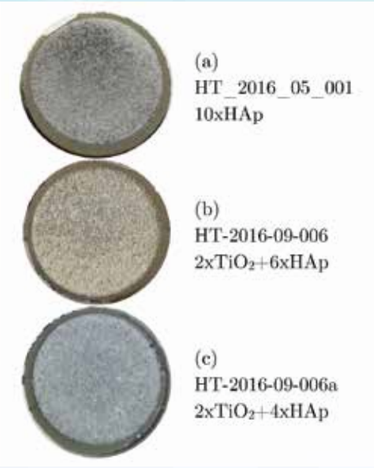


Fig. 2. Deposition difference when applying TiO2 as a bonding layer before HAp deposition. (a) No TiO2 applied as bonding layer and 10 layers of HAp, open coating in the upper left corner (b) 2 layers of TiO2 prior to 6 layers of HAp, closed coating (c) 2 layers of TiO2 prior to 4 layers of HAp, closed coating.

shown by Figure 3(b).

One important consideration when depositing HAp by thermal spraying, is to make sure that it does not decompose into undesired dehydroxylated phases due to the hot plasma jet, such as oxyhydroxylapa-

tite (OHA) and oxyapatite (OA), as well as thermal decomposition products such as tri-(TCP) and tetra-calcium phosphates (TTCP), and quenched phases such as amorphous calcium phosphate (ACP) [6].
Therefore, in order to confirm that HAp did not decompose and kept a phase mostly crystalline after high thermal exposition, an X-ray Diffraction (XRD) analysis was conducted (15°<2θ<68°, scan step time 13.77 s, generator settings 45 kV and 40 mA, CuK_{α} radiation), were the HAp spectra obtained after deposition was compared to a HAp powder spectra prior to deposition, as shown in Fig. 4. It is seen that both spectra are practically identical, demonstrating that HAp conserves its chemical structure and composition after spraying (Fig. 4(b)).As mentioned, the main goal of the study was to obtain equivalent micro-structures on the plasma sprayed polymers, when compared to plasma sprayed metal substrates reported by other authors, like Heimann [10] (Fig. 5), Demnati et al. [5] and Dey and Mukhopadhyay [6]. Thus, scanning electron microscopy (SEM) was

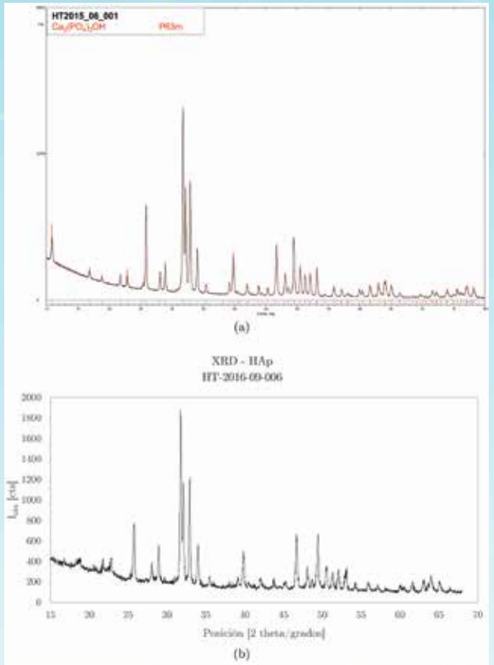


Fig. 4. HAp XRD spectra comparison: (a) HAp powder prior to deposition, (b) HAp deposited (plasma sprayed), where well defined peaks denote a phase mostly crystalline and high similarity with the spectra shown in (a).

Fig. 3. Comparison of PEEK substrates with different depositions, regarding surface preparation and deposited material adherence: (a) substrates without any treatment ("Polish"), (b) substrates treated with corundum blasting ("Rau").

carried out for different depositions and the respective images are shown in Figure 6 in.

For HAp deposition only on PEEK, Figure 6(a) shows overlapped and properly melted splats, without significant porosity. For the deposition of TiO_2+HAp, shown in Figure 6(b), properly developed splats are seen, but also some micro-porosity is exhibited, as shown by the arrow. This porosity is a product of high viscosity in the HAp, which reveals not enough enthalpy was applied in the sprayed particles for the HAp to reach the ideal molten state before impacting the surface.

Figure 6(c) displays the antibacterial deposition of TiO_2+HAp+3 wt.% Cu, where it is shown how the copper particles, in brighter contrast, were properly molten and overlapped with the HAp rich regions. In general, one can confirm the similarities of the coating SEM images with the studies on metals of

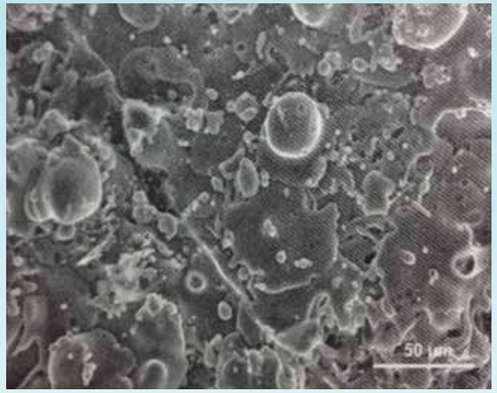
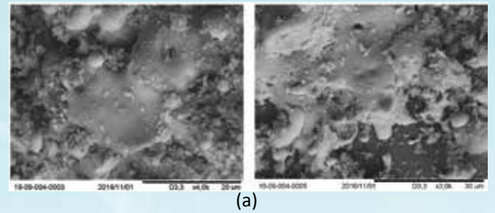
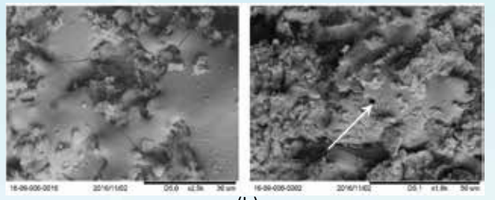


Fig. 5. Typical SEM image of plasma sprayed HAp coating, over a metal substrate, presented by Heimann [5]. The image shows well developed and overlap splats, with some loosen particles in the surface that did not reach complete melting.

the above-mentioned authors, like the one shown at Fig. 5, as well as with some of the available polymer substrates studies [8,15,16].
Regarding the coating stability tests, samples were placed in an ultrasonic bath using ultrapure water for 15 minutes. SEM imaging, is exhibited in Figure 7. Figure 7(a) shows the case for HAp deposition, Figure 7(b) the TiO_2 + HAp deposition and Figure 7(c) the TiO_2+HAp+3 wt.% Cu deposition. The images show that the coatings were stable in the three cases studied, but sharp edges and big pores, left by washed big particles, are revealed. The pores are indicated by arrows in the respective figures.

MG63 osteoblasts were cultured in PEEK samples with TiO2+HAp and TiO2+HAp+3 wt.% Cu deposition, to prove if any cell adhesion would be possible, since PEEK is a bioinert material. As it is seen in Figure 8, cells (pointed by arrows) started to grow over the HAp surface in (a) and even at the surface containing copper in (b), after just one hour of culture. This, is evidence for a successful implantation of a possible bone implant in the future.


(a)


(b)

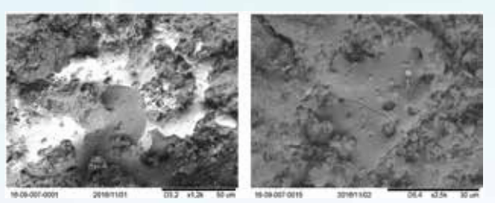
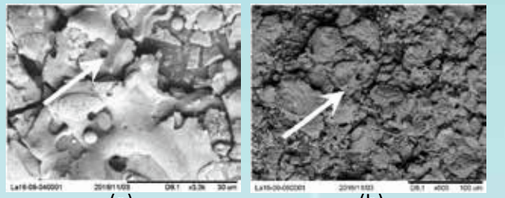

(c)

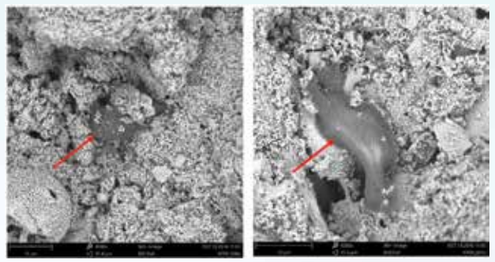
Fig. 6. (a) HAp deposition over PEEK substrate, with overlapped and properly melted splats. (b) TiO2+HAp deposition, with well-developed splats and some micro-porosity product of high viscosity HAp. (c) TiO2+HAp+3 wt.% Cu deposition, where both copper (brighter contrast) and HAp particles are well deposited.



(a) (b)

(c)

Fig. 7. Plasma spray coated samples after ultrasonic bath. (a) HAp deposition, (b) TiO2+HAp deposition, (c) TiO2+HAp+3 wt.% Cu deposition.



(a) (b)

Fig. 8. SEM imaging of MG63 osteoblasts (pointed with arrows, darker contrast) growing on PEEK substrates, coated with (a) TiO2+HAp, and (b) TiO2+HAp+3 wt.% Cu.

4. Conclusions

Overall, the results show comparable outcomes with the reported by other authors for plasma sprayed coatings over metals, proving the viability for bioactive and antibacterial plasma sprayed coatings over polymers, especially in high temperature thermoplastics like PEEK. For low melting point polymers like PVA and PLA, it is recommended to experiment with other different parameters and preferably, with a low energy plasma spray system (LEPS), to avoid substrate damage. Furthermore, the coatings over PEEK show good adherence to the substrate after a simple stability test, since they keep most of the micro-structure seen before the ultrasonic bath.

Additionally, the growth of bone cells in the samples show the clinical potential of the presented combination of bioactive coatings and polymers, opening interesting opportunities in the field of orthopedics and tissue engineering.

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Journal of Engineering in Medical Devices

DEVELOPMENT AND CHARACTERIZATION OF BIOMATERIALS FOR BIOMIMETIC TISSUE APPLICATIONS

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ABSTRACT

To create artificial tissues, a matrix is needed as structural support. It must be biodegradable, and it must cause the minimal inflammation response in the human body. However, it is important to consider the cost, which usually is very high, and the materials used: their origin and properties.
In this work, animal waste and plant extracts were used to generate a biomaterial that is part of a multiphase system from type I collagen as a temporary support, taken from rat tails; which can also be used as a nanoparticles distributor. Those nanoparticles were taken from a plant, called Tinospora cordifolia, and also from a waxy resin obtained from bee hives, called propolis, both used as regenerative agents.

It was necessary to prepare and characterize the collagen and nanoparticles using Polyacrylamide gel electrophoresis (SDS- Page), Amplitud Modulated Atomic Force Microscopy (AFM) and Thermogravimetric Analysis (TGA). Skin cells behavior with the nanoparticles was evaluated too.
Collagen obtained has a correct structural conformation, with a 65nm D-periodic and repeated sequences of triplets Gly-Pro-Pro (GPP) and a denaturalization temperature from 75 to 85ºC. Nanoparticles from natural extracts have spherical shapes, in sizes of 13.8 nm in average from tinospora and 15 nm from propolis. They produce positive effects on the regeneration of the cells in study.

The results show that it is possible to produce a biopolymeric prototype that induce cell self-regeneration from natural materials, as a useful solution in the treatment of skin diseases.

Keywords: collagen, propolis, tinospora, nanoparticles, biomaterials, biomimetic tissue.

Introduction

Sustain, restore and enhance tissue and organ functions by delivering the necessary cells and biological environments to regenerate the diseased parts, is one of the main objectives of the tissue engineering [1]. This multidisciplinary field is very recent and since it was first developed, a wide range of biomaterial scaffolds have been fabricated for tissue engineering applications [2]. Biomaterial is a systemically, pharmacologically inert substance designed for implantation within or incorporation with living systems [3] which is able to perform, replace, or enhance a natural function without giving rise to any undesirable toxic reactions to the surrounding tissues/bones [4] and scaffolds are implants or injects, which are used to deliver cells, drugs, and genes into the body [5]. Thus, various factors need to be taken into consideration to select an ideal scaffold, including chemical composition of material, its porous structural design, mechanical as well as degradation properties [4] and water adsorption, considering that water is important for maintaining the resiliency and lubrication of joint in cartilage [2].

Some of the main natural polymers that have been investigated for creating scaffolds or matrices are alginate, proteins, collagens, gelatin, fibrins, and albumin [5].

Collagen has a complex hierarchical conformation. Cell adhesive ligands such as Arginine-Glycine-Aspartic acid (RGD) aminoacid triplets are abundant in this protein and play vital roles for cellular attachment [6]. These, as well as other physical and chemical characteristics, make collagen one of the favorite biomaterials for the development of matrices and motivates the advance of this work that takes those properties, to conform a scaffold which can also be used as a nanoparticles distributor. Nanoparticles are obtained from natural sources: Tinospora and propolis.

Tinospora cordifolia, also commonly known as guduchi, is an important medicinal plant distributed throughout the tropical Indian subcontinent and China [7] and it is widely used as anti-bacterial, analgesic, antipyretic and also for the treatment of jaundice, skin diseases, anemia, etc. [8]. Currently, studies have been carried out using tinospore extracts to differentiate and regenerate the cells of the nervous system [9], or using it against tumor cells [10]. However, the use of the nanoparticles of these extracts in the tissue engineering has not yet been reported in the literature, similar to the propolis.

Propolis is a brownish waxy product collected by the honeybee from plant buds, leaves, and exudates. It is known from ancient times, possesses antimicrobial, anti-cancerous [11], antioxidant, anti-inflammatory, anaesthetic, hepato-protective, immunostimulating and cytostatic properties [12]. It is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris, but it is important to consider that this chemical composition and properties may vary depending of the geographical origin [13] and collection season [14]. In Costa Rica this natural compound is not commercialized, it is not edible, and results have not yet been reported that involve this residue with cellular regeneration. The present investigation is aimed at the obtaining and characterization of nanoparticles of tinospora and propolis, and its evaluation of cytotoxicological properties, to consider its use in natural matrices based on type I collagen, obtaining a viable and lower cost option of scaffold based on biomaterials which stimulate cell proliferation, with applications in the treatment of various dermal pathologies.

Materials and Methods

Collagen type I extraction.

Tendons were extracted from Wistar Hannover male specimens, provided by the Laboratorio de Ensayos Biológicos (LEBI) of the Universidad de Costa Rica (UCR). Approximately 1 g of tendon was solubilized on 200 ml of 3% acetic acid solution with agitation, for 24 h at 4 ºC. The solution was filtered at room temperature with gauzes and centrifuged at 4500 rpm for 30 min. The supernatant was lyophilized at 1.3 mbar, -20 ºC, for 240 h.

Collagen type I gelation.

Lyophilized collagen was dissolved in 3% v/v acetic acid solution while bringing the pH 7.47 by adding dropwise a 1 N solution of sodium hydroxide (NaOH). The solution was incubated overnight at 4 °C for gelation. The gel was centrifuged and washed three times with Type I ultrapure water and was dialyzed with a Spectra/Por 3 membrane (32 mm diameter, 3.2 ml/cm volume) shaking overnight at 4 °C during 4 d. Water was changed every 2 h. For non-dialyzed collagen, these steps were omitted. Whenever dry films of collagen were needed, the gel was dried on an oven at 45 °C during 4 d.

Tinospora nanoparticles extraction.

The stems of the plant from Varanasi, India, were sprayed and the product was added in ethanol, in agitation. Filtration (medium porosity) was performed, and a sample of this alcoholic solution was placed in the oven at 45 °C for 16 hours, obtaining the dried extract. Ultra-purified water was added to that extract. The resulting aqueous suspension was sonicated according to the conditions: power: 4W, time: 5 minutes, amplitude: 40%, probe diameter: 6mm.

Subsequently, three concentrations of the aqueous solution of tnospora nanoparticles of 10 µg/ml, 100 µg/ml and 1000 µg/ml were obtained to perform different tests.

Propolis nanoparticles extraction.

The pure propolis extract was diluted in ethanol under stirring, prior to filtration. A sample of this alcoholic solution was dripped in ultra-purified cold water with stirring during 30 min. To evaporate the ethanol the sample was placed in the oven at 45 °C. The resulting aqueous suspension was cold sonicated according to the conditions: power: 4W, time: 5 min, amplitude: 40%, probe diameter: 6 mm and then it is filtered, pore size 5 µm. Subsequently, three concentrations of the aqueous solution of propolis nanoparticles of 10 µg/ml, 100 µg/ml and 1000 µg/ml were obtained to perform different tests.

Collagen and nanoparticles characterization.

Polyacrylamide gel electrophoresis (SDS- Page). The electrophoresis assay was performed according to the methods of Laemmli [15], using 4% gel stacking and 7.5% gel resolution. Samples were dissolved in 0.5 M Tris-HCl buffer containing 25% (v / v) glycerol, 2% (w / v) SDS, 5% (v / v) β-mercaptoethanol and 0.1% (w / v) bromophenol blue. Thermoscientific molecular weight markers, 100V, were used for 2 hours. After electrophoresis, the gels were visualized with Coomassie-Brilliant Blue R-250.

Amplitude modulated atomic force microscopy (AFM). Dry films of collagen were directly deposited on the sample holders. AFM images were obtained with an MFP-3D Classic system (Asylum Research, CA), using tapping mode at room temperature.

Thermogravimetric Analysis (TGA). Thermal stability anal analysis were performed on a TA Instruments USA brand, model Q500 under inert nitrogen atmosphere, flow of 90.0 ml / min, in platinum crucibles, at a heating rate of 10 °C / min and a temperature ramp of 20 to 800 °C. Approximately 5 mg of each sample was used.

Cytotoxicity assay. For the cytotoxic evaluation, 3T3 fibroblast cell line were used, planted in a 96-well plate and growing in an incubator during 24 h, then the samples provided with the Alamar Blue compound were added, which allowed cell viability measurement. Fluorescence readings were obtained with a gain of 70 at the times: 0, 24, 48 and 72 hours with an emission and excitation of 540, 590 nm. At the mean of the data the fluorescence target was subtracted (supernatant with culture medium in the absence of cells).

Results and Discussion

Collagen characterization

To assure the quality of type I collagen according to its chemical composition the SDS-Page analysis is performed. The profile obtained in Figure 1 shows three bands: A band, on the left, indicates the protein markers with its apparent molecular weights (kDa), B band correspond to 10 µg of collagen, C band designates 15 µg of collagen and D band correspond to 20 µg collagen.

SDS-Page analysis revealed the presence of intense bands according to the molecular weights of these subunits of approximately 140 kDa and 110 kDa. It is also possible to appreciate the presence of

other bands above these subunits, close to 170 kDa. Protein patterns are similar for the different collagen concentrations used.

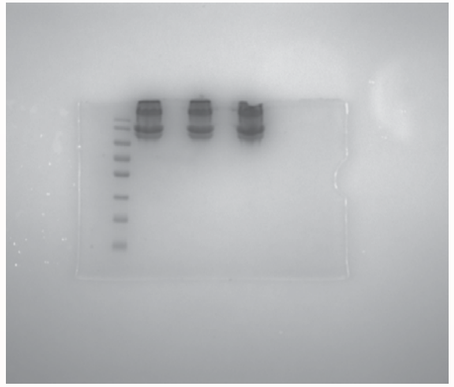

Figure 1. SDS-PAGE profile of collagen isolated from rat tail tendons, Different concentration of collagen was loaded per lane and the A band correspond to protein markers with their apparent molecular weights (kDa) indicated on the left.

The morphology of the self-assembled collagen fibers can be observed in detail, in Figure 2, with the atomic force microscopy (AFM) image.

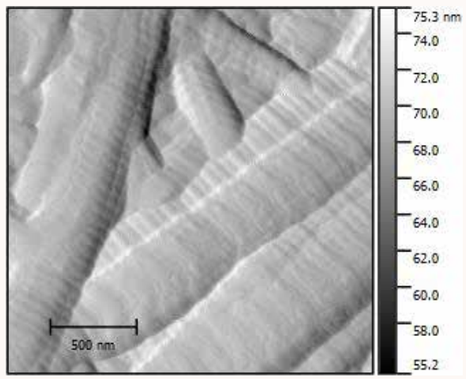

Figure 2. Atomic force microscopy (AFM) self-assembled collagen images in the amplitude mode, 10 µm.

The TGA thermograms of the different collagen samples are shown in Fig. 3. Through this analysis three important thermal processes can be observed for freeze-dried collagen (which has not undergone self-assembly processes), dialyzed (purified) collagen and no dialysis collagen. The first slight weight loss is between 25 and 150 °C, principally visible for the freeze-dried collagen, followed by a weight loss between 250 and 500°C. After reaching the temperature of 500 ° C, a slight weight loss continues, close to 10% by weight for the three samples of collagen.

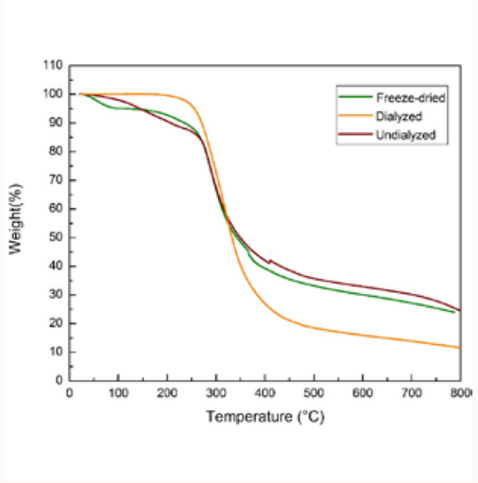

Figure 3. TGA curves of three collagen samples of 5± 0.5 mg: freeze dired, dialyzed and undialyzed, heated at 10°C/min from 25°C to 800°C in a nitrogen atmosphere.

Nanoparticles characterization

The size of the particles and nanoparticles of tinospora can be observed by electron microscopy (AFM) in Fig. 4. Particles from aqueous extract are on the left, with an average size of 42 nm, and nanoparticles after sonicating and filtering on the right, with an average size of 13.8 nm.

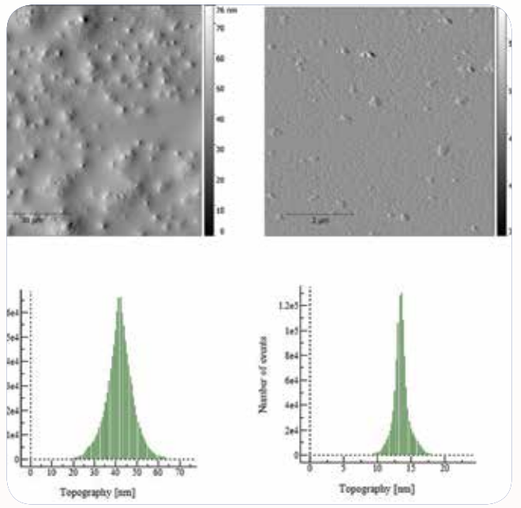

Figure 4. Atomic force microscopy (AFM) tinospora particles images on the left and nanoparticles images on the right, in the amplitude mode, 10 µm; and histogram of the particle size distribution of tinospora.

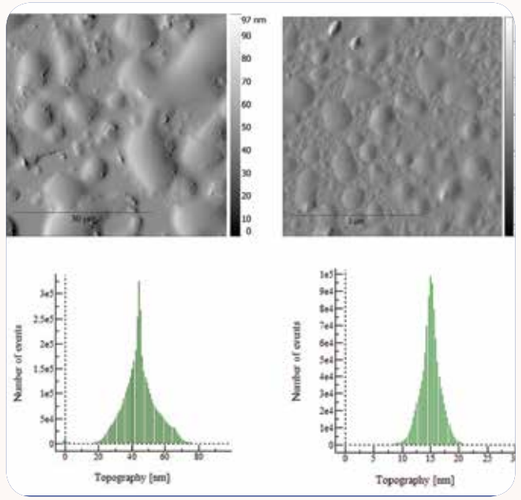

Figure 5. Atomic force microscopy (AFM) propolis particles images on the left and nanoparticles images on the right, in the amplitude mode; and histogram of the particle size distribution of propolis.

Particles and nanoparticles of propolis observed by electron microscopy (AFM) (Fig. 5) showed an average particle size of 45.8 nm and 15 nm respectively. Particles from aqueous extract are on the left and nanoparticles after sonicating and filtering on the right.
Both the tinospora and propolis nanoparticles have a spherical shape.

Cytotoxicity of tinospora and propolis nanoparticles was evaluated using 3T3 fibroblast. Evaluation of tinospora nanoparticles in different solutions (Fig. 6) showed that the nanoparticles at higher concentrations did not show significant increase in cells growth as the lower concentration did. However cell viability increases at all concentrations and times, more than 100%, except for the concentration 100 µg/ml at 72 h. From the 22 h incubation studies, it was observed that the nanoparticles at lower concentration showed the major cell viability.

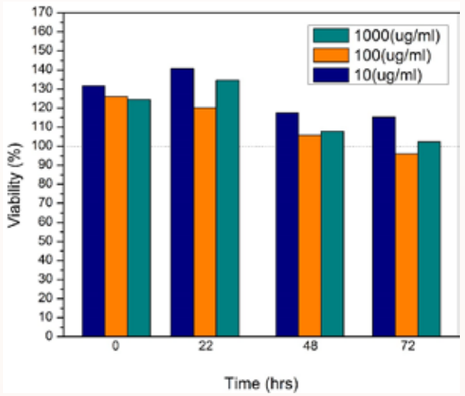

Fig. 6. Tinospora nanoparticles cell viability for three different concentration: 10, 100 and 1000 µg/ml.

In propolis nanoparticles cytotoxicity studies, there is a tendency of increase in cell viability with decreasing concentration, as seen in figure 7. Based on the control 100%, higher values (up to 141%) are obtained near the 22 hours at lowest concentration (10 µg/ml). All concentrations used for all measured times showed a viability greater than 100%.

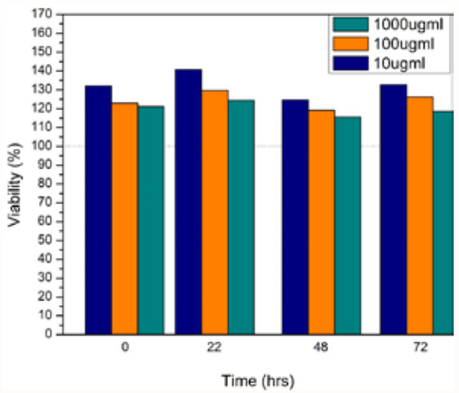

Fig. 7. Popolis nanoparticles cell viability for three different concentration: 10, 100 and 1000 µg/ml.

From the electrophoretic profiles obtained it was observed that the extracted type I collagen molecule consists mainly of two distinct subunits with molecular weights of approximately 140 kDa and 110 kDa, according to the protein marker used, which can be assigned respectively as the collagen chains α1 (I) and α2 (I), according to previous studies, and which show a polypeptide composition of this type, quite common of the type I collagen molecule between different tissues, mainly mammals [16,17]. Some α-chains were found to remain in cross-linked forms, resulting in appreciable levels of β chains (α-chain dimers) and small amounts of Y chains (α-chain trimer), also found in other type I collagen extracts [18], even in some extracted from fish skins [19]. It is observed that in all the columns, the contents of the chains are very similar to each other. It seems that the difference in the amounts of added collagen is not enough to cause noticeable changes in the intensities of the bands corresponding to the chains α, β, and Y. These data indicate an adequate collagen type I chemical composition, and the acceptable extraction process is ensured.

Collagen microfibrils form a pattern with a period (D-period) of 65 nm, a value that is within the characteristic values of 64-67 nm [20] and corresponds to the displacement of each molecule in the axial direction, with Relative to the adjacent molecule [21]. It is argued that this variation in periodic spacing is intrinsic to type I collagen, its self-assembly, is a reflection of local changes in mechanical stresses and may be the result of the variant in intrafibrillary interactions including hydrophobic, electrostatic, Hydrogen bonds and crosslinks in hydroxylysines and hydroxyproline [22].
The diameter of each fibril has a measurement of between 450 and 550 nm, which coincides with that reported for tendon fibrils that are up to 1 cm long and 500 nm in diameter [23].

About collagen thermal stability, the first decrease in percentage of significant weight that is presented in TGA is related to a structural water loss, between 25 and 150 °C, mainly for freeze-dried collagen, which is highly hygroscopic [24]. The second weight loss observed in a temperature range that is between 250 and 500 °C is due to the decomposition of collagen molecules and depolymerization [25], once all the water bound to the collagen is released; Then, after reaching the temperature of 500 °C, a slight loss close to 10% by weight for the three collagen samples corresponding to the combustion of the residual organic components continues [16,24].
For the latter degradation, close to the temperature of 600 °C, residues were quantified for lyophilized, dialysed and dialysed collagen of approximately 30.08%, 15.97% and 32.91%, respectively. The mass loss of the collagen without dialysis is lower, which shows a greater thermal stability for it, which is associated to the presence of sodium acetate salt residues in the structure of this collagen, that has not been purified; as opposed to dialyzed collagen, for which a loss of 84.03% of mass is reported, the greater with respect to the other collagens, which implies that there is less organic residues in it, thus evidencing the effectiveness of the process of collagen purification employed.

When analyzing the size and morphology of extracts and nanoparticles of tinospora and propolis, it can be observed that in the aqueous extract of both tinospore and propolis, no particles with well-defined shapes are distinguished, whereas once this solution is subjected to the sonication and filtration process the formation of nanoparticles can be clearly seen, with even more defined spherical geometry.

The average size of the nanoparticles of tinospora is approximately 13.8 nm, according to the histogram with a geometric distribution of the data and the average size of the nanoparticles of propolis is 15 nm. The values obtained for the dimensions of the propolis particles agree with values reported in other studies: approximately 50-170 nm, using methods of suspension of the alcoholic extract on water, similar to the methodology used in this study, to obtain the hydroalcoholic extract [11]. In spite of the good results obtained, it is recommended to perform dynamic light scattering studies to corroborate the size of the hydrodynamic diameter of the nanoparticles.
The effectiveness of the nanoparticle synthesis method (sonication and filtration) is evident when comparing the sizes obtained from the aqueous solutions.

3T3 epithelial cells were used to evaluate cytotoxicity of nanoparticles because they reside in the connective tissue and are able to synthesize fibers and maintain the extracellular matrix of the tissue of many animals [26]. The results suggest that cells metabolic activity increases significantly in the presence of the tinospora and propolis nanoparticles, reinforcing some current studies [8,27]. The lowest concentration for all the times increased the cell viability more than 100% in both, tinospora and propolis.
Data obtained also revealed the potential of tinospora and propolis nanoparticles, as regenerative agents in biomimetic tissues.

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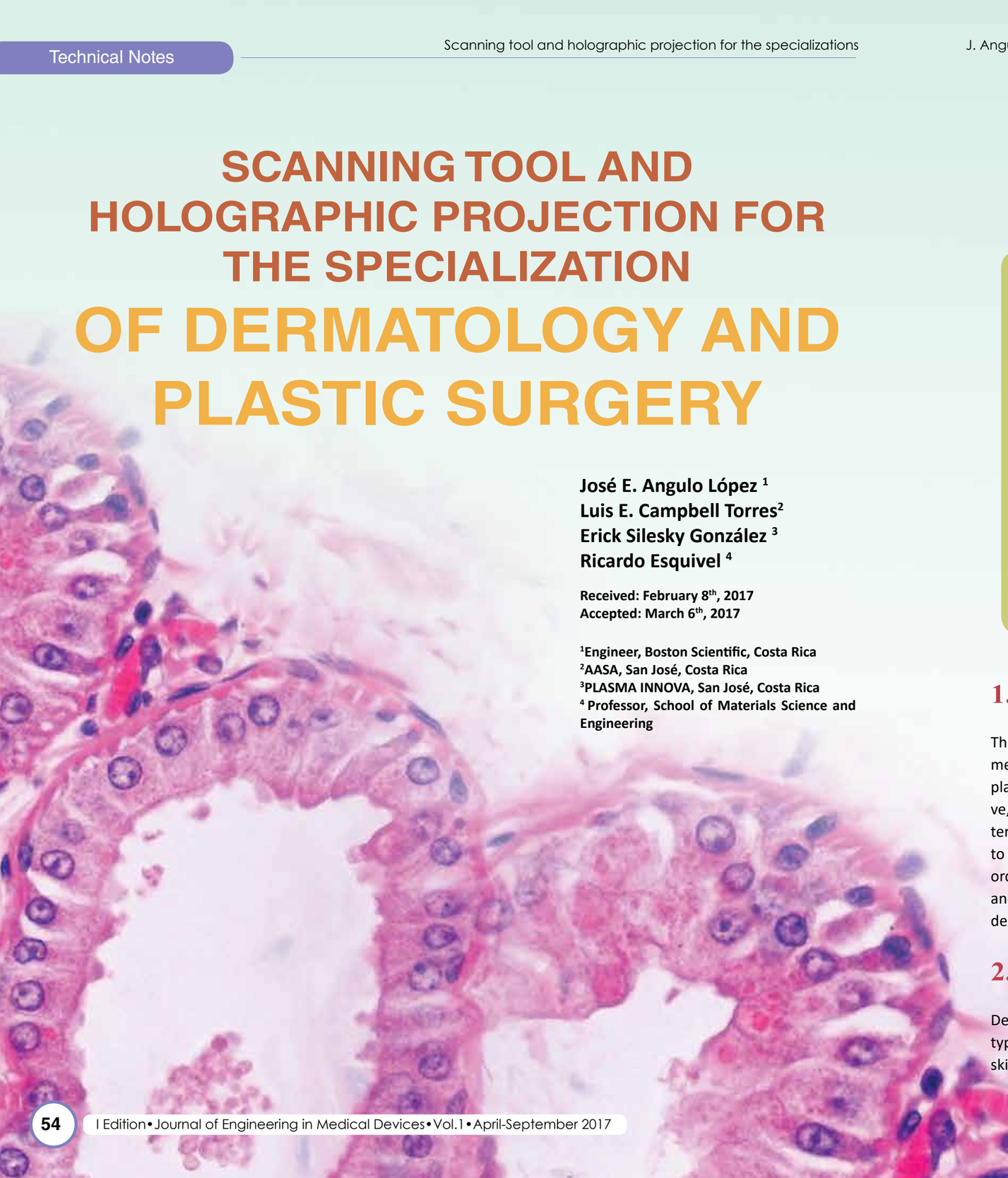
Journal of Engineering in Medical Devices

SCANNING TOOL AND HOLOGRAPHIC PROJECTION FOR THE SPECIALIZATION OF DERMATOLOGY AND PLASTIC SURGERY



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ABSTRACT

Skin cancer is one of the fastest growing diseases in the world. Early detection and treatment are key to reducing mortality caused by this disease, especially in its most lethal form (melanoma). However, the criteria for diagnosis of skin lesions is diffuse, subjective, and is based more on factors like experience of the doctor and specific situations, rather than quantifiable and truthful criteria. This project seeks to provide a scientific alternative detection to these methods, by designing a device capable of mapping the skin tissue, based on technology available in the marketing that ensures repeatability and traceability of the processes of detection.

Interviews with experts in the field of dermatology and vision systems, as well as a literature survey related to the study area, agree that although there are advances in scanning and digitizing of skin lesions, there is still a way to go to be able to obtain repeatable data that can be used for diagnosis. The proposed device seeks to further advance technology in this area.

Keywords: ***skin cancer, melanoma, dermatology, plastic surgery, detection, classification, scanning, repeatability, traceability.***

1. Introduction

This paper describes the need for a device to support medical areas of dermatology and reconstructive plastic surgery in order to carry out more quantitative, scientific assessments with less variability of criterion possible, which in turn counts with the ability to properly store the results of tests performed, in order to use the results for subsequent comparisons and evaluations of possible progress of the patients' dermatological diseases.

2. General objective

Design a system with the ability to detect different types of skin cancers such as melanoma and other skin related diseases.

3. Specific objectives

Perform quantitative studies of the skin, in relation to its topography, appearance, color, and changes in general.
Determine in the physiognomy of the human skin.
Develop detailed specifications and construction schematics, for manufacturing the resulting device.

4. Justification

Melanoma cancer is one of the rarest types of cancer but it represents the highest number of cancer death in the world [1-3].

During the past four decades this type of disease has increased by 200% in the United States of

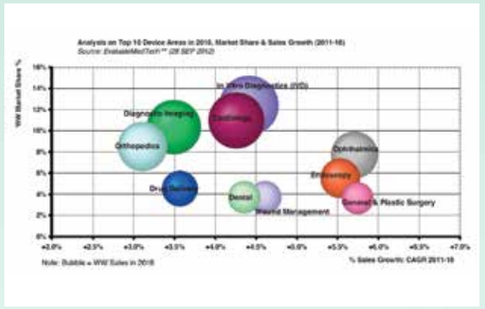

Figure 1: Analysis of Top 10 areas of medical devices for 2018

America and in the same way there have been similar increases detected in other regions such as Europe [3].

In 2015 there were more than 73 000 [2] new cases of this type of cancer detected, from these about 9 900 people died from this disease only in the United States of America, where the death rate is probably lower compared to other latitudes because of the great technological medical advances available in that region.

In some parts of the world, especially the Western World cities, melanoma has become a fairly common disease. According to the World Health Organization, around the world about 132 000 new cases of melanoma are diagnosed each year [4]. This incident is aggravated in caucasian populations that are generally located in lower latitudes, with the highest incidents in countries like Australia, where there are almost twice as much of this type of diseases compared to European cities.

Currently, dermatologists do not have a tool that allows the implementation of quantitative studies that support qualitative assessments made by doctors on the condition of the skin. Nowadays, there is no device capable of making comparisons between exams made to patients. On the contrary, everything is based mainly on subjective judgment of the doctor, hindering the early detection and prevention of skin diseases.

The device shall have the ability to perform, store and compare studies to be made to a person, giving specialists a tool in order to get accurate, fast and assertive decisions and diagnoses benefiting the patient being treated.

In the international context during the last three years, the global trend of medical investment in the area of diagnostic imaging occupies one of the top three places in amount of investment as shown by Figure 1.

5. State of the Art

According to Linden [1], the incidence of malignant melanoma is increasing more than any other type of cancer. To minimize or reduce mortality rates, an appropriate and early detection is imperative. Early diagnosis of this disease is something that is becoming more and more important to save human lives. With the advancement of technology, tools have been developed to diagnose skin cancer more quickly and efficiently, however there are certain deficiencies as indicated by Ogorzalek [2] Despite all these developments there is still a wide margin for misinterpretations of images related to skin lesions. Additionally, the ability to detect malignant skin lesions varies depending on the physician [3,4]; and except for people at high risk or a history of skin cancer, there is a debate about the frequency of medical check-up, who should do the check and who should get it.
The possible methods to diagnose cancer in an early manner have been discussed extensively. In 2000 there was the second meeting of consensus on public opinion research and its main conclusions were that four algorithms, namely, pattern analysis, ABCD rule, Menzies scoring method and the checklist of 7 points are good ways to evaluate skin lesions. All four methods share common concepts and allow the selection of specific features that are possible to perform with the help of the computer "(Orgalek et al, [2]). This information in particular and the study mentioned by Linden [1], it is concluded that developments in imaging will help to the detection and diagnosis of skin cancer and in the long-term allow live detection and increased certainty of the detection and analysis of skin cancer. These are fundamental basis of the current research, and are expressed as a starting point for the work shown in this report.

As for technologies that help detect the skin surface, there are two main types: scanners and digitizers [5]. Scanning is the process by which images in three dimensions are converted to digital form by using optical or video tools; meanwhile, digitization is to trace and document the three-dimensional shapes on a computer. There are also dynamic and static systems depending on how they take images. The dividing line between the different categories is slightly blurry, with the emergence of new technologies in recent years, however it works as the foundation for the next section, which deals with the steps needed for image capture and processing for 3D scanning.

Capture and image processing

A detection system for skin cancer or melanomas is typically composed of some basic processes as: image acquire, processing, segmentation of lesions, feature extraction and classification. The first stage of the system relates to the process of how the image is taken, generally through photographs or from a previously existing physical record. The segmentation of lesions found is the main process of the detection system, because it defines the area considered prone or affected by cancer or melanoma. Once segmented the region of interest, it is possible to submit it to comparisons and studies such as type of edges, color, topography, changes in size, among others, in order to be compared with those algorithms previously exposed.
In relation to the detection method in which the current research is based, a technique called "ABCD rule" is used [6,7]. It has been decided to use this technique, because it is standardized and its potential to be used with a technology that captures in 3D. This technique involves obtaining the diameter and height of the injury, usually overlooked, because most dermatologic images have no reference points to determine and compare the sizes of lesions. Once the characteristics of the lesion are determined and measured, image processing continues and leads to the classification by the treating physician.

Design elements to consider

Based on what was exposed in the previous sections, it was decided and define the design to focus the project to address the main problems currently found on the tools of image capturing, and beyond designing the method or deal with the capture algorithm, submit a practical and economical solution using components available on the market. Therefore, the research is based more on the subtlety of integration, and how to fill a market gap.

To this end, it has been decided to use as part of the device a high resolution camera with capabilities of optical coherence tomography (OCT) [4] of 5 layers to be able to represent a three-dimensional injury. OCT technique provides an assessment by a cross section cut, and the imaging taken by layers, of the epidermis and dermis. It allows the analysis of collagen and other physical characteristics of the skin [8]. This could help solve some challenges

during image acquire, such as uneven illumination, as shown in the example of Figure 2. Taking into account the experience of the company in the area of visual inspection for different production and quality processes, a prototype has been developed already, as shown in Figure 3.

As for the challenge of segmentation of images [9], the thresholding method has been selected, which is based on calculating the thresholds in the black and white images, based on pixel density values in the images that generate the inspection, as shown in an example in Figure 4. Finally, and as option tool for discretionary use by the treating physician, an algorithm is going to be used, which aims to take pictures previously stored in a database, to be able to make comparisons with injuries previo usly analyzed, related or based on a method called Linear Discriminant Analysis (LDA) [10].

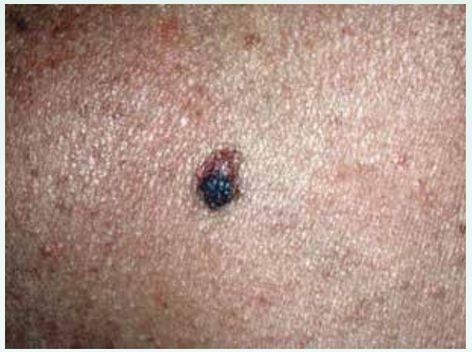

Figure 2: Uneven illumination.

Figure 3: Funcional Prototipe

Figure 4: Thresholding method

6. Conclusions

• The technology presented is meant to make more accurate diagnosis of the dermal state, helping in choosing the most appropriate treatment for each client.

• Increasingly, the skin analysis based on principles scientifically recognized are more valued, since they have many advantages over visual analysis performed by a specialist.

• The equipment designed allows the identification of skin lesions using cameras that achieve sharper and better quality images as well as being suitable for all types of contextures according to your design.

• The built prototype enables professionals in dermatology, scan, detect, select, play and compare studies performed on patients.

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