CHAPTER 1

Underwater pulsed ultrasound in animal model osteochondral injury

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ABSTRACT
Objective: To study changes in an osteochondral surface, submitted to a standardized mechanical lesion analyzing the use of pulsed ultrasound with a frequency of 1MHz, at 20%, with frequency modulation of 16Hz, 0.5 W/cm2 of intensity, and application time of 5 minutes in these lesions. Method: Sixteen white, male, New Zealand rabbits (*Oryctolagus cunicullus)* less than six months old, with weight ranging from 1,540 grams (g) to 2,450 with standardized lesions in femoral condyles were used. They were submitted to daily application of pulsed ultrasound at a frequency of 1MHz, at 20%, with frequency modulation of 16Hz, 0.5 W/cm2 intensity and application time of 5 minutes in two groups with two and four weeks of osteochondral lesion. Result: The animal model selected and the formatting of the histological analysis were adequate for the proposed research. The use of pulsed ultrasound with frequency of 1MHz, at 20%, with frequency modulation of 16Hz, 0.5 W/cm2 of intensity and application time of 5 minutes in acute osteochondral lesions showed similar statistical significance in chondral regeneration compared to the Control Group. In the application of USPSA (UNDERWATER PULSED ULTRASOUND) in the proliferative phase (third and fourth week) of acute osteochondral lesions there was a decrease in osteoblastic proliferation and formation of immature bone. Conclusions: There was an improvement in osteochondral regeneration with the use of the method, but ultrasonic emission parameters applied in the treatment of acute osteochondral lesions must continue to be studied, as these presented here constitute only one of the numerous therapeutic possibilities of this modality.

Keywords: osteochondral lesion, underwater pulsed ultrasound.

1 INTRODUCTION

Methods of restoration and replacement of joint defects represent a major contribution of medicine to the 20th century[1] , and it is stated that surface restoration is fundamental to the use of the joint in a physiologically appropriate manner[2] .

The greatest difficulty is not to diagnose the cartilaginous lesion, because both clinically and through complementary examinations the destruction of the articular cover can be identified and classified. It is the cure or the best management of the lesion that brings apprehension to the therapist.

In today's society it is estimated that 60% of people over the age of 35 report symptoms of joint pain, and the deterioration of joint structure not only decreases quality of life but also has a significant economic impact, especially as the population of a country ages[3] .

There is a strong association between joint degeneration and age. In some populations, 60% to 90% of people over the age of 65 have been shown to have osteoarthritis, compared to less than 5% of people between the ages of 15 to 44 years and 25% to 30% of people between the ages of 45 to 64 years. Authors have calculated that 95,000 total knee arthroplasties and 41,000 other procedures to repair cartilage defects are performed each year in the United States of America (USA) .[4]

In Brazil, the prevalence of osteoarthrosis is 16.19%, responsible for 30 to 40% of consultations in rheumatology outpatient clinics, according to data from the INPS/INAMPS, and is also the cause of 7.5% of all work absences. It is the second disease among those that justify initial assistance with 7.5% of the total, second also in sickness assistance (in extension) with 10.5% and fourth in determining retirement with 6.2%[5] .

Although the cause of osteoarthritis is often unknown and there is no cure for it, early diagnosis and treatment can help minimize symptoms and help patients maintain an active life.

Despite the frequency and impact caused by degenerative joint disease, it is being considered as an inevitable consequence of aging. Research is always looking for ways to slow the progression of osteoarthritis in more diverse ways .[6]

Several experimental studies have been conducted in order to clarify the different aspects related to joint biological responses[7,8] .

Recently, the use of ultrasound applications has been reported as a resource capable of promoting the restoration of cartilaginous articular surfaces[9] , but the literature has not yet presented elements capable of supporting such statements on an objective and consistent basis.

2 OBJECTIVE

The present work aims to evaluate whether the application of underwater ultrasound has a positive effect on the restoration of injured articular cartilaginous surfaces.

PATHOPHYSIOLOGY OF JOINT INJURIES

Changes in Cartilage

Physiologically the self-repair of articular cartilage comes from the intermediate zone being an interactive process that adds the biomechanical adhesive property to a subordinate molecular cellular healing process[11] .

During chronic degeneration, the scarring process produces, for superficial deposition, type I collagen associated with fibronectin and tenacin forming fibrocartilage, a structure that is stiffer than the original cartilage[12] . The closest visible signs of osteoarthritis are localized fibrillation or disruption of the most superficial layers of articular cartilage (Blade 5).

As the disease progresses, more parts of the joint surface become wrinkled and uneven, and the fibrillation extends deeper into the cartilage until it reaches the subchondral bone.

When the cracks in the cartilage grow, the superficial edges of the fibrillated cartilage wear away, releasing fragments and decreasing the thickness of the cartilage. At the same time, enzymatic degeneration of the matrix can further decrease the volume of the cartilage. Eventually, the progressive loss of articular cartilage leaves the bone exposed (Blade 5).

Many of the mechanisms responsible for the progressive loss of cartilage remain unknown, but the process can be divided into three overlapping phases: that of cartilage damage, that of chondrocyte response, and that of the decline in chondrocyte response.

In the first phase, the macromolecular frame matrix breaks down and the aqueous content increases. The concentration of type II collagen remains constant, but decreases in aggregation and proteoglycan concentration generally accompany the increase in aqueous content. These changes increase the permeability and decrease the stiffness of the matrix, changes that may increase its vulnerability to further damage. Chondrocytes detect tissue damage, possibly as a result of changes in osmolarity, charge density, or pressure applied across the cell membrane, tethered to the matrix molecules.

Once the chondrocytes detect tissue damage, they release mediators that stimulate the tissue response. Nitric oxide likely plays a role in this response, since chondrocytes produce this molecule in response to a variety of mechanical and chemical stresses. Nitric oxide diffuses rapidly and can induce the production of IL-1, which stimulates the expression of metalloproteases that degrade matrix macromolecules. Fibronectin fragments of IL-1 or other molecules from damaged tissue can promote IL-1 production. Degradation of type IX and XI collagens and other molecules can destabilize the fibrillar cross-linkage and type II collagen, allowing aggregate expansion and increasing water content. A decline in aggregation, and in particular an associated loss of aggrecan, could increase pressures on the remaining collagen fibril network and chondrocytes, with joint overload. Enzymatic degeneration also eliminates damaged matrix components and may release anabolic cytokines, previously encased in the matrix, that stimulate synthesis of matrix macromolecules and chondrocyte proliferation. Shells or clones of the cells, which proliferate surrounded by the newly synthesized matrix molecules, constitute one of the signs of the chondrocyte reparative response to cartilage degeneration. This response counters the catabolic effects of proteases and can stabilize or, in some circumstances, even restore the tissue.

The failure to stabilize or restore the tissue leads us to the third phase of degeneration, namely the decline in chondrocyte response, which may be irreversible. This decline may result from mechanical damage or death of chondrocytes long unprotected and stabilized by the functional matrix, but this also appears to be related to or initiated by down-regulation of the chondrocyte response to anabolic cytokines. It may occur as a result of synthesis or accumulation of molecules in the matrix that bind to the anabolic cytokines.

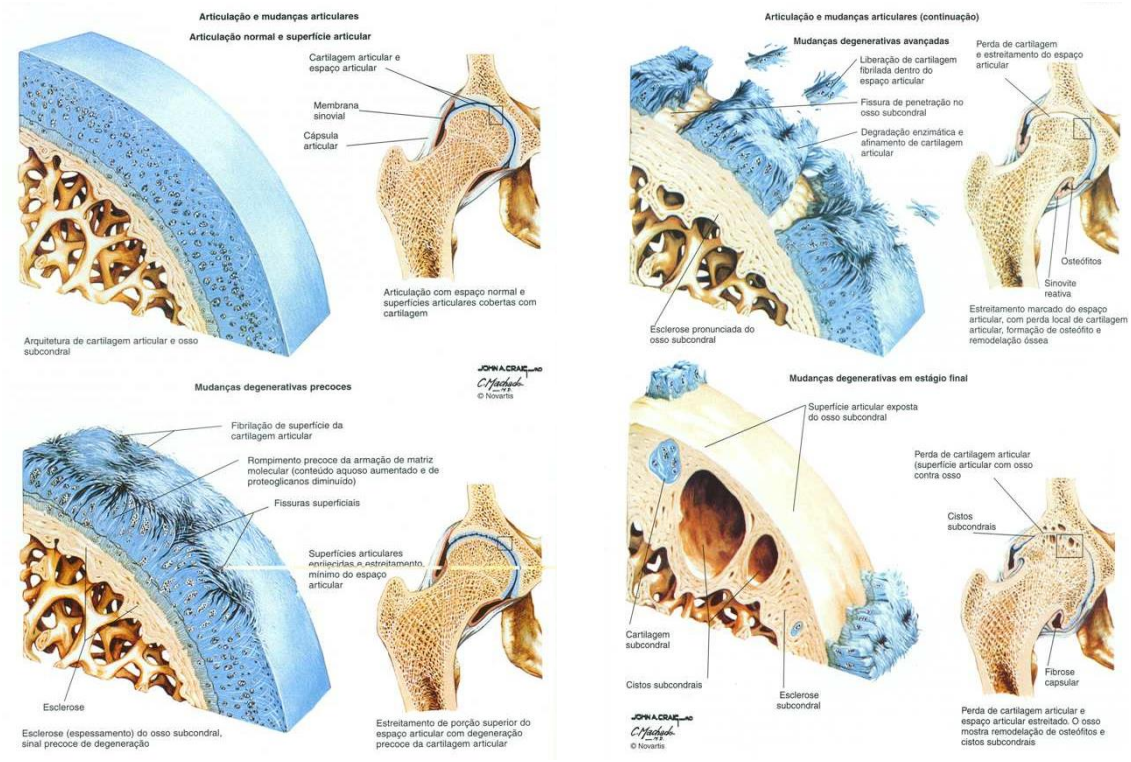
The injury and reparative response is distributed into three classes[13] :

1- Cartilage damage, but with intact surface (internal chondral damage and possible subchondral damage)
2- Mechanical tearing of the articular surface limited to the thickness of the cartilage
3- Mechanical tearing of articular cartilage and subchondral bone

An epidemiological study, describes that subchondral sclerosis determines changes in bone replacement further accelerating the osteoarthrosis process[14] .

The described evidences lead us to believe that any experiments that are performed, aiming to restore articular surfaces, must have them previously injured, under penalty of not having a local situation similar to the one that would be presented by an injured joint.

Figure 1 - Articulation and Articular Changes



PHYSICAL PRINCIPLES OF ULTRASOUND

WAVES

Ultrasound is a form of mechanical energy that consists of high-frequency vibrations. Ultrasonic waves are longitudinal and cause oscillations in the particles of the medium in which they propagate. The frequencies of ultrasonic waves range from 20,000 to 20,000,000 cycles per second (1 cycle/second = 1 Hertz) and are inaudible to human hearing, whose sensitivity ranges from 20 to 18,000 Hz.

The medical frequency for diagnostic imaging ranges from 5,000,000 to 20,000,000 Hz, i.e. 5 to 20 megahertz (MHz), and the therapeutic from 0.7 to 3 MHz.

WAVE PRODUCTION

Ultrasound was originally produced by a quartz crystal vibrating when subjected to a high frequency current discovered by LANGEVIN in 1917. This vibration drives the particles in the medium, producing

waves by compression and decompression called the piezoelectric effect. Any material that transforms one energy into another is called a transducer.

Currently we have synthetic ceramic crystals as transducers, and the metal alloy composed of lead, zirconium, and titanium (ZTC = Zirconate-titanate of lead) is the most commonly used for its durability and efficiency in converting electric current into mechanical vibrations.

EFFECTS OF ULTRASOUND ON INJURY REPAIR

if used correctly and at the appropriate time after injury has occurred, ultrasound can be a very powerful therapeutic modality. "Correctly" means using the lowest intensity possible to obtain the desired result (intensities above 1 W/cm2 should not be necessary), and the "appropriate time after injury occurs" means during the inflammatory phase of repair. Clinicians should take advantage of the numerous wound assessment techniques that currently exist in order to test the efficacy of their therapies[15] . Finally, ultrasound can be dangerous if employed incorrectly; thus, users should fully understand the mechanisms of how this modality works.

It is proven active in the Inflammatory, Proliferative and Remodeling stages of chondral lesions[9] .

3 MATERIAL AND METHODS

MATERIAL

Sixteen white, male rabbits (*Oryctolagus cunicullus),* less than six months old, of the New Zealand lineage, with weight varying from 1,540 grams (g) to 2,450 g, were used. They came from and were housed at the Coelhário do Colégio Agrícola Federal Senador Gomes de Oliveira (CAFSGO) in Araquari, Santa Catarina, in an agreement with the Universidade da Região de Joinville-UNIVILLE.

The rabbits were kept in metal cages measuring 0.81 meter (m) X 0.60 m X 0.45 m, with a maximum of three per cage, in a protected environment, isolated from noise, fed with food suitable for the species and water at will.

The choice of breed was only made for greater homogeneity of the sample, as well as the choice of males to avoid hormonal variability and, in the case of mixed housing, crossbreeding.

The animals were submitted to veterinary follow-up and operated on in a specially built surgical environment under specific standards of disinfection and asepsis.

We followed the Animal Ethics Protocol governed by Federal Law 6.638/79

The instruments used to make the lesion were designed by Biológica-GMReis Campinas - Brazil and the ultrasound equipment used in the study was the AVATAR V model from KLD Amparo - Brazil. The collected material was sent for histological analysis.

4 METHODS

The procedures were carried out in four distinct steps:

PROCEDURE 1 (ONE) performing the surgeries to produce the osteochondral lesions according to Schueda et al's Animal Model .[16]

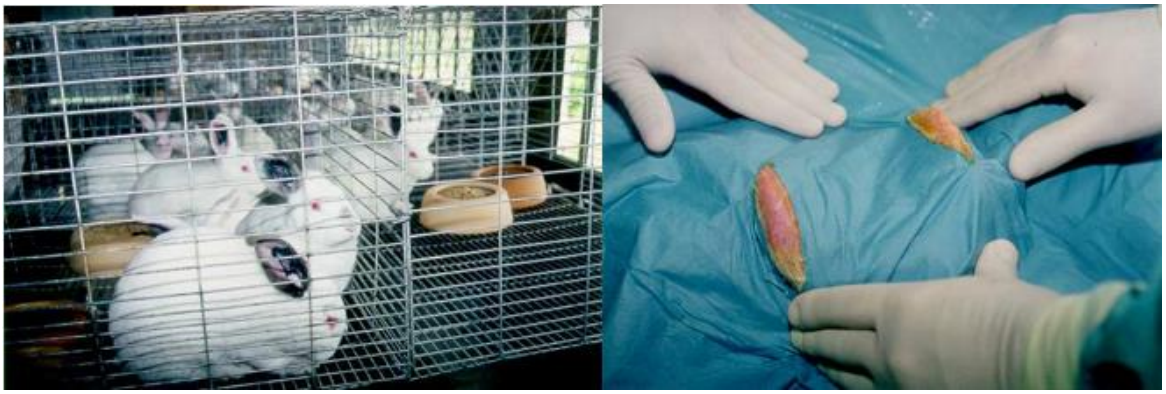
PROCEDURE 2 (TWO) Application of SUBAQUATIC PULSED ULTRASOUND in the Animal Model in groups A and B.

PROCEDURE 3 (THREE) Collection of material for analysis.

PROCEDURE 4 (FOUR) Histological analysis.

All procedures from preoperative, anesthetic induction, anesthesia, antisepsis, surgery, immediate and late postoperative care as well as euthanasia were performed in the Experimental Surgery Laboratory of CAFSGO. The rabbits were maintained with water and appropriate feed at will until the moment of the operation (Figure 2).

Figure 2 - Photograph of the rabbit room with conditioning of the animals and procedure on knees after anesthesia and placement of fields.



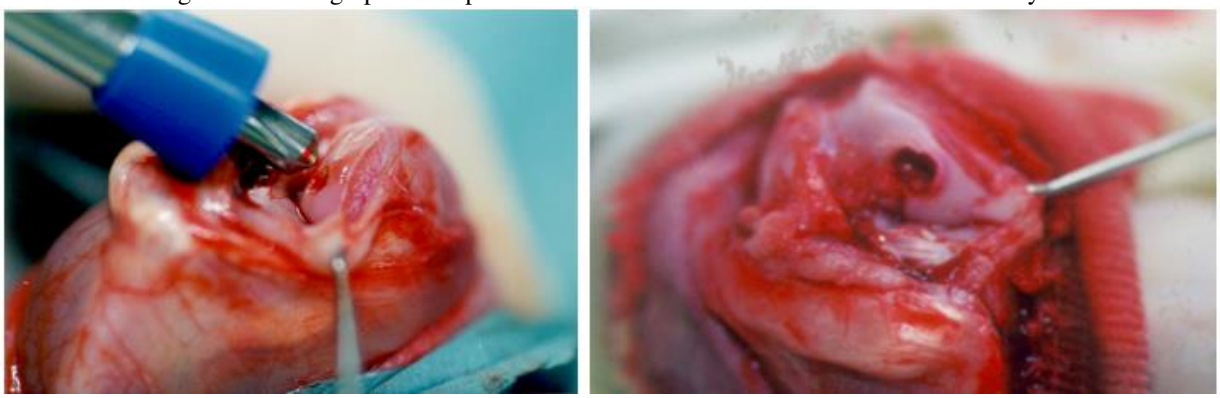
PROCEDURE ONE

After weighing, the animals were submitted to pre-anesthetic medication with acepromazine 1%, intramuscular (IM) in the *régio glutea* (gluteal region), at a dose of 0.2 milligrams (mg) per kilogram (kg) of body weight ($0.2mg.kg^{-1}$).

After fifteen minutes they received, as anesthetic medication, xylazine $10mg.kg^{-1}$ and ketanine $25mg.kg^{-1}$, by the same route and place of the pre-anesthetic medication.

The standard procedure was followed, producing the osteochondral lesions (fig. 3) and then closing the surgical wound by layers.

Figure 3 - Photograph of the production of the lesion in the Medial Femoral Condyle.



PROCEDURE TWO

The 16 operated rabbits were divided into two groups of 8 animals:

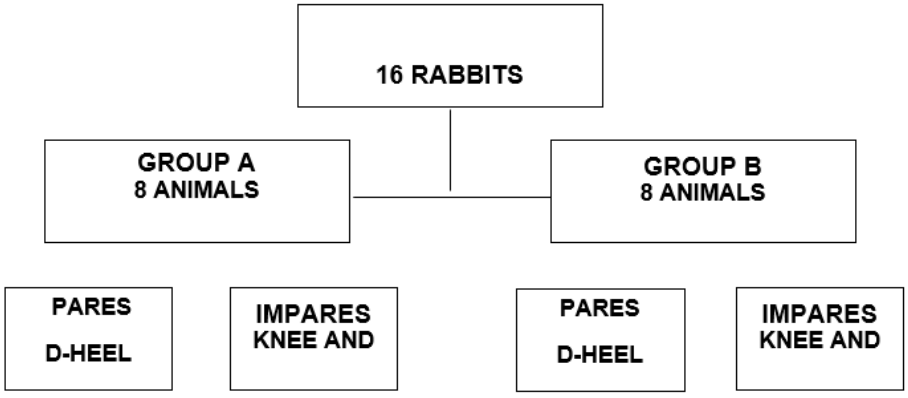
GROUP A - Application of SUBAQUATIC PULSED ULTRASOUND (USPSA) in the first two weeks postoperatively with a daily application.

GROUP B - Application of SUBAQUATIC PULSED ULTRASOUND in the third and fourth postoperative weeks with a daily application.

The groups were subdivided in such a way that the Peer rabbits received USPSA application on the Right knees (D) while the Unpeers received it on the Left knees (E) (Organogram 1).

The untreated knees remained as Control Group

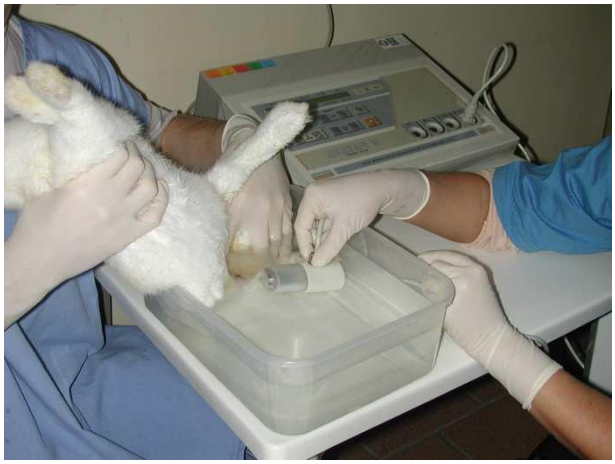
Chart 1 - Distribution of the groups and subgroups for the study.



Based on the previous theoretical foundation, the frequency, intensity, mode, duration, and treatment interval were formatted through calculations of tissue thickness in the rabbit knee region. The parameters used in the equipment were: frequency of 1MHz, 0.5 W/cm² intensity, pulsed mode at 20% with modulation frequency of 16Hz and application time of 5 minutes, totalizing 10 applications.

Indirect contact was chosen as the ultrasound application technique, through the underwater way (Figure 4).

Figure 4 - Photograph of underwater pulsed ultrasound application.

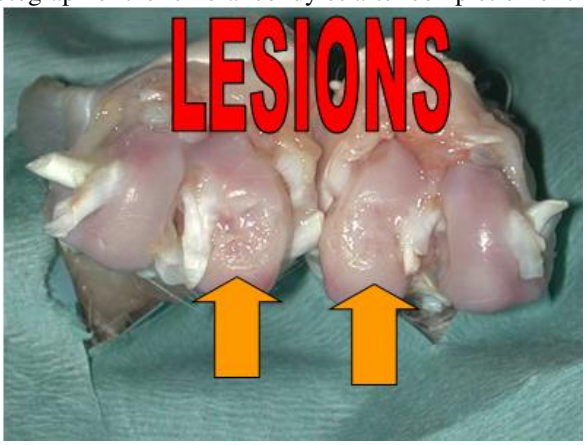


PROCEDURE THREE

After the second week in Group A and after the fourth week in Group B, the animals were euthanized with an intravenous injection of 3 milliliters (ml) of 10% potassium chloride.

The knees were exposed using a number 15 scalpel blade and after total removal of soft tissue, right and left femoral supracondylar osteotomy was performed with an oscillatory saw (Figure 5).

Figure 5 - Photograph of the femoral condyles after completion of the experiment.



They were placed in identified flasks with 10% formaldehyde (Figure 30) and sent to the CEDAP Laboratory (Centro de Diagnóstico Anatomo Patológico) for the preparation of blocks, slides and histological analysis of the samples with sections tangential to the lesions.

PROCEDURE FOUR

Hematoxylin and Eosin (HE) and Masson's stain were used to analyze the samples.

All slides were analyzed by two experienced histologists, who answered the predetermined questionnaire, and these professionals did not know the Group to which the slides belonged.

STATISTICAL EVALUATION

The variables were represented by absolute (n) and relative (%) frequency.

The comparison between the two groups regarding the distributions of the

The proportions of YES or NO answer was done by the Chi-square test. The

Differences were located by the Chi-square Partition test.

The two groups were compared to the distribution of scores using the Studant's T method. A significance level of 0.05 (a = 5%) was adopted, and descriptive levels (p) below this value were considered significant and represented by *.

5 RESULTS

HISTOLOGICAL ANALYSIS

GROUP A - treated in the first two weeks

Control Group

Histological analyses of the injured condyles in Group A (Fig. 6) revealed the presence of different amounts of hyaline cartilage and fibrocartilage irregularly distributed throughout the lesion area. They also reveal immature bone, osteoblastic proliferation and other regenerative changes described above. There is an invasion of healthy tissues around the injured area, but no complete regeneration of the osteochondral architecture neighboring the lesion.

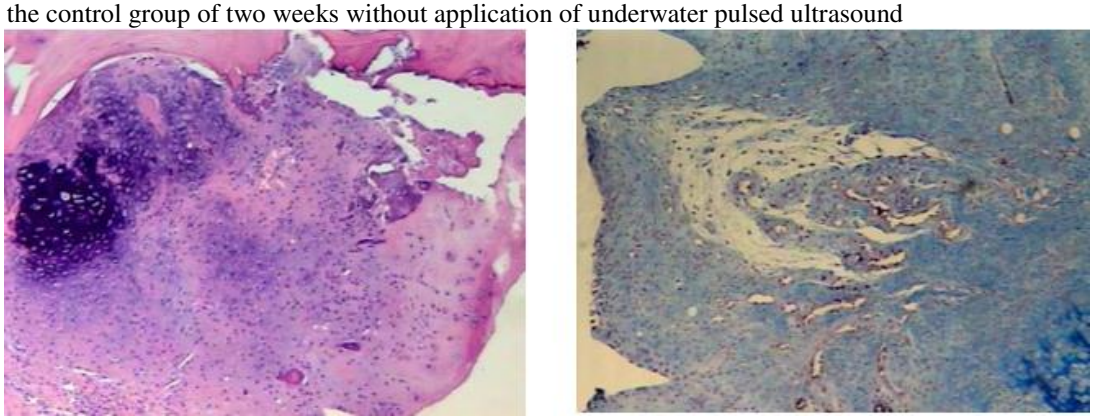
The different tissue regenerations organized in Table I were measured in a semi-quantitative way.

Masson's staining differentiated proportionally the amount of hyaline and/or fibrous cartilage present.

The intensity of immature bone tissue formation and osteoblastic proliferation, cartilaginous tissue as well as subchondral vessel formation and remodeling were quantified.

The scar response of structuring a chondrogenic layer, cartilaginous matrix and chondrocyte nests were grouped together and assessed as to whether or not they appeared in a summation of signs.

Figure 6 - Photomicrographs at 40x magnification of H&E stained MASSON stained knee cartilage sample from animal belonging to the control group of two weeks without application of underwater pulsed ultrasound



With application of pulsed underwater ultrasound

Microscopic examination of the samples taken from Group A revealed the presence of different amounts of hyaline cartilage and fibrocartilage irregularly distributed throughout the lesion area. Similarly, there is an invasion of healthy tissue around the lesion area. By applying Masson's staining, the amount of existing hyaline and/or fibrous cartilage and the other histological alterations present were differentiated proportionally (Fig. 7).

Figure 7 - Photomicrography at 40x magnification of a knee cartilage sample stained by H&E and by MASSON from an animal belonging to group a with two weeks of underwater pulsed ultrasound application

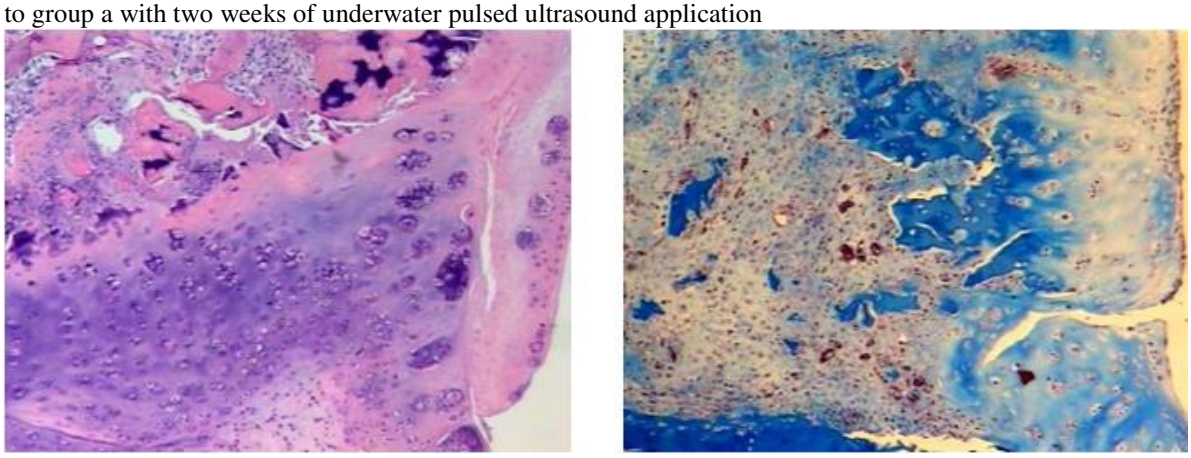


Table I was associated with the evaluation of the group of knees submitted to the application of ultrasonic treatment.

Table 1 - Distribution of histological alterations found in group A

0-2 WEEKS W/US	01	02	03	04	05	06	07	08	AVERAGE
FIBER REACTION	1	1	4	1	4	2	2	1	2
HYALINE REACTION	4	4	1	4	1	3	3	4	3
IMMATURE BONE	3	2	4	2	3	4	4	4	3,25
PROLIF. OSTEOB.	4	2	4	4	4	4	4	3	3,625
C.C.+ M.C.+ N.C.	4	4	1	4	4	4	4	3	3,5
VESSELS	3	2	2	2	2	3	2	2	2,25
REMODELING	1	1	2	2	4	2	3	4	2,125
0-2 WEEKS WITHOUT	01	02	03	04	05	06	07	08	AVERAGE
FIBER REACTION	3	1	1	3	4	1	4	3	2,5
HYALINE REACTION	2	4	4	2	1	4	1	2	2,5
IMMATURE BONE	2	2	4	2	4	2	2	2	2,5
PROLIF. OSTEOB.	2	3	4	3	4	3	3	3	3,125
C.C.+ M.C.+ N.C.	4	2	4	4	4	4	2	4	3,25
VESSELS	3	3	2	4	2	2	3	3	2,75
REMODELING	3	1	2	4	1	3	3	4	2,625

Comparison between Groups with and without USPS application on treated lesions in the first two weeks

Group A with and without application were compared (fig. 8), considering the presence of fibrocartilage, hyaline cartilage, and other scar parameters.

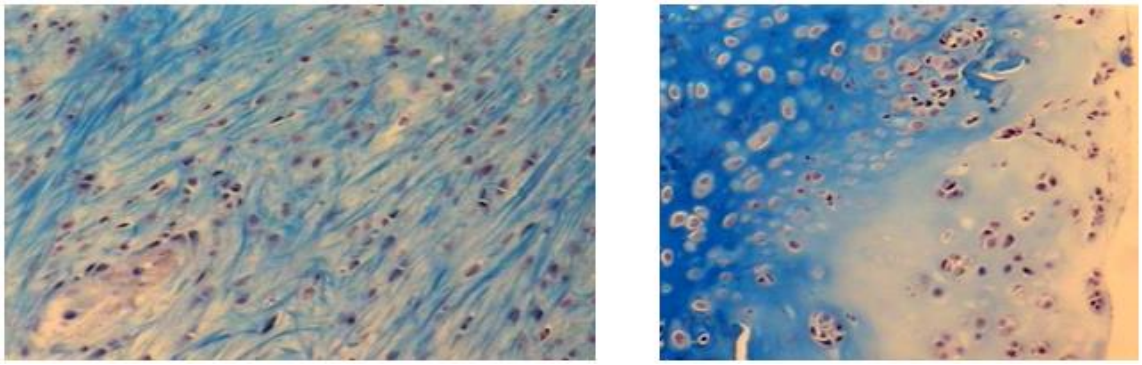
Group B - Treated at the third and fourth week post injury

Without applying underwater pulsed ultrasound

The microscopic examination of the samples taken from Group B revealed the presence of different amounts of fibrocartilage, hyaline cartilage (Fig. 8) distributed irregularly throughout the lesion. There was invasion of healthy tissue around the injured area, but no complete regeneration of the osteochondral architecture surrounding the lesion.

By applying H&E and Masson's staining, the amount of hyaline and/or fibrous cartilage formed was differentiated proportionally.

Figure 8 - Photomicrography at 100x magnification of a Masson-stained knee cartilage sample from an animal belonging to group B without and with application of underwater pulsed ultrasound showing area of fibrocartilage



The intensity of immature bone tissue production, osteoblastic proliferation, cartilaginous tissue, as well as subchondral vessel formation and remodeling, were quantified as shown in the table above.

The scar response of chondrogenic layer structuring, cartilaginous matrix and chondrocyte nests were grouped and assessed as to whether or not they appeared in a summation of signs.

By collecting data from the group, we formatted Table 2, which evaluates the responses from the site subjected to the injury.

Table 2 - Distribution of histological alterations found in group B

3-4 WEEKS WITH US	1	2	3	4	5	6	7	8	AVERAGE
FIBER REACTION	3	4	1	4	4	3	1	4	3
HYALINE REACTION	2	1	4	1	1	3	4	1	2.125
IMMATURE BONE	2	3	2	2	2	2	3	2	2.25
PROLIF. OSTEOB.	3	3	2	2	2	2	3	2	2.375
C.C.+ M.C.+ N.C.	3	1	4	1	1	4	4	3	2.75
VESSELS	3	4	3	2	3	3	3	4	3.125
REMODELING	2	4	1	3	2	2	3	1	2.25
3-4 WEEKS WITHOUT US	1	2	3	4	5	6	7	8	AVERAGE
FIBER REACTION	4	4	4	3	1	1	3	4	3
HYALINE REACTION	1	1	1	2	4	4	2	1	2.375
IMMATURE BONE	4	3	4	3	3	2	3	2	3
PROLIF. OSTEOB.	4	3	4	3	3	3	3	3	3.25
C.C.+ M.C.+ N.C.	4	1	1	2	4	4	4	2	2.75
VESSELS	3	4	4	2	2	4	3	3	3.125
REMODELING	2	4	2	4	2	3	2	2	2.625

With application of pulsed underwater ultrasound

Microscopic examinations of the fragments taken from the group reveal the presence of different amounts of hyaline cartilage and fibrocartilage irregularly distributed throughout the lesion area. Similarly, there is an invasion of healthy tissue around the injured area. Masson's staining was applied to differentiate proportionally the amount of existing hyaline and/or fibrous cartilage and the other histological alterations present.

Table 2 shows the evaluation of the group of knees submitted to ultrasonic treatment.

STATISTICAL ANALYSIS OF GROUP A - TREATED IN THE FIRST TWO WEEKS

	FIBROUS REACTION W/ US	*FIBROUS REACTION W/O US*
AVERAGE	1,75	2,5
VARIANCE	1,071428571	1,714285714
REMARKS	8	8
STANDARD DEVIATION	1,0034556	1,027316256
	HYALINE REACTION W/ US	*HYALINE REACTION W/O US*
AVERAGE	3,25	2,5
VARIANCE	1,071428571	1,714285714
REMARKS	8	8
STANDARD DEVIATION	1,0034556	1,027316256
	IMMATURE BONE W/ US	*W/O US IMMATURE BONE*
AVERAGE	3,25	2,5
VARIANCE	0,785714286	0,857142857
REMARKS	8	8
STANDARD DEVIATION	0,988014305	0,992322093
	PROLIF. OSTEOB W/ US	*PROLIF. OSTEOB W/O US*
AVERAGE	3,625	3,125
VARIANCE	0,553571429	0,410714286
REMARKS	8	8
STANDARD DEVIATION	0,970864639	0,956482416
	C.C+M.C.+N.C. W/ US	*C.C+M.C.+N.C. W/O US*
AVERAGE	3,5	3,5

VARIANCE	1,142857143	0,857142857
REMARKS	8	8
STANDARD DEVIATION	1,006698908	0,992322093

	VESSELS W/ US	*W/O US VESSELS*
AVERAGE	2,25	2,75
VARIANCE	0,214285714	0,5
REMARKS	8	8
STANDARD DEVIATION	0,925869251	0,965936329

	REMODELING W/ US	*W/O US REMODELING*
AVERAGE	2,375	2,625
VARIANCE	1,410714286	1,410714286
REMARKS	8	8
STANDARD DEVIATION	1,017353663	1,017353663

IN NONE OF THE ABOVE TESTS WERE THERE SIGNIFICANT DIFFERENCES BETWEEN THE MEANS *P>0*.05. STATISTICAL TEST USED: studant's *t*.

GROUP B - TREATED IN THE THIRD AND 4TH WEEKS

	FIBROUS REACTION W/ US	*FIBROUS REACTION W/O US*
AVERAGE	3	2,875
VARIANCE	1,714285714	1,553571429
REMARKS	8	8
STANDARD DEVIATION	1,027316256	1,022272225

	HYALINE REACTION W/ US	*HYALINE REACTION W/O US*
AVERAGE	2	2,125
VARIANCE	1,714285714	1,553571429
REMARKS	8	8
STANDARD DEVIATION	1,027316256	1,022272225

THERE WERE NO SIGNIFICANT DIFFERENCES BETWEEN MEANS *P>0*.05. STATISTICAL TEST USED: studant's *t in* the above tests

	IMMATURE BONE W/ US	*IMMATURE BONE W/O US*
AVERAGE	2,25	3
VARIANCE	0,214285714	0,571428571
REMARKS	8	8
STANDARD DEVIATION	0,925869251	0,972407047

THERE WAS SIGNIFICANT DIFFERENCE BETWEEN THE MEANS *P>0*.05. STATISTICAL TEST USED: studant's *t*.

	PROLIF. OSTEOB W/ US	*PROLIF. OSTEOB W/O US*
AVERAGE	2,375	3,25
VARIANCE	0,267857143	0,214285714
REMARKS	8	8
STANDARD DEVIATION	0,936257181	0,925869251

THERE WAS SIGNIFICANT DIFFERENCE between means *p>0*.05. Statistical test used: t-student.

	C.C+M.C.+N.C. W/ US	*C.C+M.C.+N.C. W/O US*
AVERAGE	2,625	2,75
VARIANCE	1,982142857	1,928571429
REMARKS	8	8
STANDARD DEVIATION	1,03480078	1,033384127

NO SIGNIFICANT DIFFERENCE BETWEEN MEANS *P>0*.05. STATISTICAL TEST USED: studant's *t*.

	VESSELS W/ US	*VESSELS W/O US*
AVERAGE	3,125	3,125
VARIANCE	0,410714286	0,696428571
REMARKS	8	8
STANDARD DEVIATION	0,956482416	0,982073131

THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN THE MEANS *P>0.*05. STATISTICAL TEST USED: studant's *t*.

	REMODELING W/ US	*REMODELING W/O US*
AVERAGE	2,25	2,625
VARIANCE	1,071428571	0,839285714
REMARKS	8	8
STANDARD DEVIATION	1,0034556	0,991278054

THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN THE MEANS *P>0.*05. STATISTICAL TEST USED: studant's *t*.

6 DISCUSSION

Despite humanity's great technical and scientific advances until the beginning of the 21st century, an ideal and definitive treatment for cartilage injuries has not yet been found. For more than three centuries, authors, such as Hunter since 1743, have cited the difficulties in the healing of articular cartilage. In all eras there has been, and still is, interest in the study of cartilage regeneration, a tissue of vital importance, because when injured, the main symptom is pain, and the limitation may lead to functional loss, leading many individuals to abandon their work or sports activities, regardless of age. experimental studies, with several species of animals, show the regeneration of hyaline cartilage after injuries, depending on the injured site and the size of the injury[18,19,20] .

In the Control Group analyzed by us, there was no healing of the injured areas; we only found a small percentage of hyaline cartilage in the peripheral region of the lesion. Whenever the subject of cartilage injury was approached, we thought of a probable hypothesis about the existence of some compensation mechanism of load transfer to the non-injured compartment, in an attempt to "save" the injured side. once the cartilage is injured, there is no load transfer; therefore, it is necessary to search for an effective treatment of this area[21] . In our study, since we only injured one condyle, we did not expect this transfer to compromise the uninjured cartilage.

Experimental studies are fundamental for us to understand all the stages of repair or regeneration, and rabbits are the animals of choice for several authors due to their ease of handling and low aggressiveness, being, for these reasons, an animal that offers no risks, besides being extremely easy to be anesthetized[19] .

The choice of a single breed was made for greater homogeneity in the sample, as well as the choice of males avoided hormonal variability and crossbreeding in the case of mixed housing.

The care is more related to the drugs used. Ketamine was used as an anesthetic, agreeing with several authors[22,23,24,25] for the safety of the drug in maintaining the anesthetic plan that was good for the duration of the experiments. Acetpromazine 1%, a phenothiazine tranquilizer, a potent neuroleptic, was used in all animals with expected results obtained[26] . We used the association of ketamine and xylazine, such

association provides a safe anesthesia in the animals as soon as it is administered, ending its effect in time for the performance of the procedures[27] . It enabled a good surgical time for a low level of discomfort to the animal .[22]

All animals survived the anesthesia and surgery procedures.

In an animal model, to produce a lesion that does not regenerate spontaneously, besides compromising the basal layer, it must reach the subchondral region. Its diameter must exceed 3 mm to 4 mm[20.28,29,30] . Our Model adopted this care[16] . The rabbits operated on were less than 6 months old because the durability of the reparative scar in these animals decreases with their maturation . [31]

We preferred that our rabbits remained without immobilization to effect the regenerative potential of active joint mobility, as well as operated both knees could not restrict the use of the injured[32] .

The histological exams proved that the lesion had a recent regenerative character, showing moderate structural disorganization. The chondrocytes were arranged in sparse groups, irregularly distributed in the middle of the hyaline matrix, in agreement with observations by authors[19] .

It provided a regenerative lesion, according to the animal model chosen, leaving a joint with an osteochondral lesion prepared for the desired therapeutic test, with good articular exposure, after laterally dislocating the patella, as shown in other studies[25,33,34] .

We used a semi-quantitative and gradual histological scale of cartilage repair, by means of sagittal histological sections, thus being able to graduate the filling of the lesions[35] . We modified in our histological analysis the regeneration qualification because we needed more accuracy in measuring the osteochondral response to lesions being or not stimulated through the application of electrotherapy, as we would analyze different regenerative reactions.

Based on theoretical basis, the ultrasound equipment model AVATAR V of the company KLD Biosistemas was used, as it is micro processed, meets the international and national safety standards, INMETRO and ISO 9001 certification. It offers a variety of frequencies (1 and 3 MHz) and a head with 1, 3 and 5 cm of ERA (effective radiation area), meeting the requirements of this study. The underwater application technique is considered the best for presenting similar acoustic impedance to the soft tissue, reducing reflection.

Due to the depth of the lesion we opted for a frequency of 1MHz. The intensity used was 0.5 W/cm2, for the treatment of acute lesions using low intensities, since the high ones could be harmful to the tissues. The pulsed mode was chosen for also being indicated in the treatment of acute lesions, for presenting minimized thermal effects, and the work cycle in 20% with frequency modulation of 16Hz contemplated the use in both groups.

The application time of 5 minutes has been standardized so that the treatment time is at least 1 minute for an area of 1 cm2. Areas no larger than the size of the head should be treated for a few minutes (3 to 5) using the semi-static method. The frequency of daily treatment was chosen, because in acute cases

they must be treated daily. The period of ten days is due to the fact that this is the minimum number of sessions performed in physical therapy services.

Pulsed ultrasound has a significant therapeutic effect in stimulating tissue regeneration and bone tissue repair.

In our study that analyzed chondral behavior with USPSA using 0.5 W/cm2 / 1 MHz the cartilaginous response found showed similarity in the results of the treated group and the control group in the first weeks.

The same authors found that if the treatment was delayed until the third or fourth week, the ultrasound would stimulate chondrogenesis. In our study there was statistical similarity between the groups with respect to all measurements of chondral production at the third and fourth weeks. This was confirmed in our study by the decrease in immature bone formation and osteoblastic proliferation in the USPSA treated group.

Our model, which investigated hyaline or fibrous chondral regeneration, found a tendency to form hyaline cartilage over fibrocartilage in knees submitted to USPSA therapy, but did not have statistical correspondence. And in our study of osteochondral region, the vascular proliferation parameter did not show statistical significance.

The presence of differentiated organization in terms of remodeling as well as by the presence of chondrocyte layer, cartilaginous matrix and chondrocyte niches were similar in both Group A and Group B with and without the application of USPSA.

7 CONCLUSIONS

The animal model selected and the formatting of the histological analysis were appropriate for the proposed research.

The use of pulsed ultrasound with a frequency of 1MHz, at 20%, with frequency modulation of 16Hz, 0.5 W/cm2 of intensity, and an application time of 5 minutes in acute osteochondral lesions showed similar statistical significance in chondral regeneration compared to the Control Group.

In the application of USPSA in the proliferative phase (third and fourth weeks) of acute osteochondral lesions there was a decrease in osteoblastic proliferation and immature bone formation.

The parameters of ultrasonic emission applied in the treatment of acute osteochondral lesions should continue to be studied, as these presented here constitute only one of the numerous therapeutic possibilities of this modality.

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