Self-Repair in Degenerative Joint Disease

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Abstract: This study presents a method for treating and structurally improving articulations affected by degenerative joint disease (DJD). The focus of this analysis is on two groups of patients: the first comprised patients over eighty years old, and the second comprised patients aged 45 to 55 years. The first group was a high surgical risk and both had been nonresponders to current conservative therapies.

Scholars like Davis, Filatov, and Cerletti have been studying and using the regenerative properties of placenta, amnios and other nonvital tissues since the early 1900s. These pioneering studies have opened a new track for tissue renewal. More recently, the new biological knowledge about extracellular nucleic acids, growth factors (GF) (as by-products of trauma response), and heat shock proteins (Hsp) has helped research even further.

Building on those experiences, we have developed a regenerative gel obtained with distressed, processed blood, polydeoxyribonucleotides (Pdrn), and a thickening substance. The objective was to stimulate the local innate stem cells with our gel in order induce tissue repair.

From 2003 until 2009, we treated 948 patients. As mentioned, the first group comprised of 86 ultra-octogenarian patients with severe osteoarthritis (OA) of the hip and/or knee, and the second group comprised of 90 younger patients (around 50 years old) affected by the same disease.

Treated patients have been clinically and radiologically evaluated with a follow-up of 6 to 48 months. Results show a statistically significant improvement in terms of pain and joint mobility, sometimes coupled with clear improvement in radiological imaging. Follow-up shows encouraging data in terms of clinical stability over time. During the study, we encountered virtually no side effects, adverse reactions, or toxicity.

Currently the pharmacological treatment of DJD is palliative, though toxicity and side effects of the drugs remain problematic. Patients who can be operated on could continue their trial with a prosthesis followed by a long rehabilitation period. This study presents a new methodological approach to the treatment of DJD based on tissue regeneration and restoration resulting in a positive clinical resolution.

Keywords: Degenerative joint disease (DJD), innate joint stem cells, mesenchymal stem cells (MSCs), growth factors (GF), heat shock proteins (HSP), polydeoxyribonucleotides (Pdrn), regenerative medicine, self-repair; tissue renewal.

INTRODUCTION

History

Regenerative medicine based on cell therapy and tissue engineering is a newly emerging multidisciplinary field. This type of therapy is aimed at maintaining, restoring, or enhancing tissue and organ function [1].

In 1910, Davis was the first to describe the use of the fetal membrane as a surgical support material in skin transplantation at Johns Hopkins Hospital. Since then the uses of amniotic membrane in surgery have expanded, from treatment of skin wounds to burn injuries, chronic leg ulcers, ocular surface disorders (corneal or conjunctiva disease) and prevention of post-surgical adhesion formation [2].

Nonviable amniotic membranes (amnios) treated under high-temperature sterilization were used for medical applications from the 1960s to the 1980s. During this period, the membranes did not contain viable cells and the biological properties were thus unidentified. Amnios so prepared were used therapeutically for burn injuries and chronic leg ulcers as a biological dressing [3-5].

Filatov (1875–1956) investigated what happens to tissues before death or under significant biological stress. He kept fresh placenta tissue and amnios at very low temperatures for several days, before application as a biological dressing. Filatov was convinced that not only does placenta tissue have reparative properties, but so also does any tissue put under extreme stress before dying. He thought that these biological activities were enhanced by “tissue suffering,” with the release of bio-humoral substances that he named “bio-stimuline” [6, 7].

Cerletti (1877-1963), a renowned neurologist and the “father of electroshock,” came to a similar conclusion after experimenting with electroshock and stress induction on pigs. In his experiments, Cerletti put pigs through high-voltage electroshock, extracted their brain matter, and in-
jected it to another set of pigs. The second group of animals could tolerate the electro-stimulation much better than the control group. Moreover, he noted that by taking a sample of brain extracts obtained from pigs that underwent electro-shock and injecting it into pigs made comatose by electro-shock treatment, he induced a temporary state of awakening. The experimental evidence convinced Cerletti that a tissue put under extreme stress conditions enhances the production of substances he called "acroagonine," able to induce a powerful biological reaction [8, 9].

In the 1980s Vishakarma and Khare reported their experiences using nonviable amniotic membranes to restore articular tissues damaged by degenerative or post-infectious arthritis. They reported that 25 patients out of 28 underwent arthroplasties and were free of symptoms, with a good range of movement, and a stable joint. But the most important achievement was the structural regeneration of the articulation as shown during a follow-up X-ray [10].

Back in the 1960s, R. Di Nicola began to use nonviable amniotic membranes, which were at that time on the market under the name Amniex®. The preparation of the amniotic membranes included high-temperature sterilization. He began to insert Amniex® under the skin of symptomatic arthritic patients with great clinical success. He soon noted the absence of side effects and the long-lasting efficacy of the therapy in terms of pain and mobility. Additionally, the complete clinical resolution of the disease was sometimes associated with structural joint improvement, as was highlighted by conventional imaging.

At the same time and with similar therapeutic indications, placental extracts were in clinical use for a wide range of pathologic conditions of connective tissue, such as dystrophic skin, scarring, and treatment of skin ulcers. Placental extracts were a mixture of polydeoxyribonucleotides (Pdms), compounds with polymers of different lengths, between 50 and 2000 base pairs and thermo-stable.

**Extracellular Microenvironment and Tissue Renewal**

**Role of Extracellular Nucleic Acids**

Initially, the wound-repairing effects of Pdms have been associated with its ability to induce the formation of platelet-fibronectin complexes [11, 12].

Several researchers have recently shown the importance of extracellular nucleotide and nucleoside activity, inducing proliferation and activity of cells in different tissues. Nucleic acids, nucleosides, and nucleotides derived from cell fragmentation, physiologically diffuse into the extracellular environment as a result of cell lysis following cellular death, possibly providing a local stimulus for tissue regeneration [13-17].

It has been demonstrated in vitro that nucleotides and nucleosides act as growth promoters for fibroblasts, osteoblasts and endothelial cells [18-23]. Similar results have been obtained in vivo in experimental models, using polydeoxyribonucleotides (Pdms) to stimulate healing in gamma radiation-induced lesions in mice [24].

Pdms are the product of enzymatic cleavage of nucleic acids following cell death and cell lysis. Nucleotides (like ATP) and nucleosides released by enzymatic separation work in different ways: they can stimulate nucleic acid synthesis through the salvage pathways, and their effect is mediated by binding to purinergic receptors (P1-P2).

Thellung [25] has shown that, when stimulated by a variety of nucleosides, A2 receptors (a P1 subclass) induce proliferation in cultured fibroblasts. Sini and Nakamura [26, 27] have demonstrated in vitro the Pdrn stimulatory effects on fibroblast and osteoblast proliferation and collagen production, which promote an increase in fibroblast growth of about 20%. Similarly, in vitro and in vivo studies have shown the role of A2 receptors, stimulated by nucleosides, in wound-healing processes [28].

It has been demonstrated that nucleic acids diffused in an extracellular environment act in synergy with different growth factors like PDGF, FGF, EGF, TGF-β, cytokines, growth factor production; they even influence immunologic responses [14, 15, 18, 29, 30].

Some researchers have studied the biological process that, in bone regeneration, provides an osseo-inductive signal for the mesenchymal host precursor cell, inducing proliferation and differentiation into active osteoblasts. The molecules mainly implicated in this biological process are the bone morphogenetic proteins (BMP) and other growth factors like PDGF, FGF, IGF, and TGF-β [19, 21, 22, 31].

Guizzardi has analyzed the effect of Pdrn on osteoblast growth and alkaline phosphatase production. His study has shown that the polymerization process of Pdms produces the highest levels of free purine nucleotides and nucleosides. These molecules bind to purinergic receptor A2 and, moreover, they can activate other mechanisms like the salvage pathways. The result is an increase of osteoblast growth that reached its maximum after 6 days and then decreased when the cultures became confluent. The final finding is an increased cell growth of 21% [32].

Recently he has even evaluated, in an experimental study, the positive effects of heat deproteinate bone matrix and polynucleotides (Pdrn) on bone regeneration in rat [33].

**Role of Trauma Necrosis and Hypoxia**

Surgical trauma induces some modification in the biological microenvironment that is often capable of inducing basic reparative transformation. Products of necrosis and O2 deficiency lead to increased production and activation of local GFs. Specific microenvironmental cues regulate self-renewal and differentiation capabilities. Oxygen is an important component of the cellular microenvironment, serving as both metabolic substrate and signaling molecule. Oxygen has been shown to have a variety of effects on embryonic and adult stem cells. The role of hypoxia in regulating stem cell biology, specifically focusing on growth, maintenance of pluripotency, differentiation, and production of growth factors is becoming more and more relevant [34].

Trauma necrosis and hypoxia cause a local increase in GFs. Some of these GFs, such as VEGF, FGF 1-2, TGF-β1 are particularly important. In fact, they can promote an increase in vascular permeability and endothelial cell proliferation, fibroblast chemotaxis, proliferation angiogenesis, and matrix deposition.
Heat shock proteins (HSP) are a class of functionally related proteins whose expression is increased when cells are exposed to elevated temperatures or other stress [38]. This increase in expression is transcriptionally regulated. The dramatic upregulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor [39].

The mechanism by which heat shock (or other environmental stressors) activates the heat shock factor has not been determined. However, some studies suggest that an increase in damaged or abnormal proteins brings HSPs into action. Consequently, the heat shock proteins are also referred to as stress proteins and their upregulation is sometimes described more generally as part of the stress response [40].

Heat-shock proteins are named according to their molecular weight. So Hsp60, Hsp70, and Hsp90 (the most widely-studied HSPs) refer to families of heat shock proteins on the order of 60, 70, and 90 kilodaltons in size, respectively [41]. The small 8-kDa protein ubiquitin, which marks proteins for degradation, also has features of a heat shock protein [42].

The fundamental function of HSPs has expressed as upregulation in stress. Production of high levels of heat shock proteins can be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exercise, exposure of the cell to toxins (ethanol, arsenic, trace metals and ultraviolet light, among many others), starvation, hypoxia, or water deprivation [40].

**Role of Heat Shock Proteins**

Heat shock proteins (HSP) are a class of functionally related proteins whose expression is increased when cells are exposed to elevated temperatures or other stressors (e.g., exercise, exposure of the cell to toxins, such as infection, inflammation, exercise, exposure of the cell to toxins, ethanol, arsenic, trace metals and ultraviolet light, among many others), starvation, hypoxia, or water deprivation.

**Function as Chaperone**

Heat shock proteins function as intracellular chaperones for other proteins. They play an important role in protein-protein interaction and maintenance, such as folding, assisting in the establishment of proper protein conformation, and prevention of unwanted protein aggregation. By helping to stabilize partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell [43, 44].

**Housekeeping**

Heat-shock proteins also occur under nonstressful conditions, simply monitoring the cell's proteins. Some examples of their role as "monitors" are that they carry old proteins to the cell's "recycling bin" and they help newly synthesized proteins fold properly.

These activities are part of a cell's own repair system, called cellular stress response or heat-shock response.

**Immunity**

Extracellular and membrane-bound heat-shock proteins, especially Hsp70, are involved in binding antigens and presenting them to the immune system [45].

The functions of HSP, which are typically associated with stress response and tolerance, are well characterized in differentiated cells, whereas their role in stem cells remains unclear.

Self-renewal and differentiation of stem cells are tightly regulated processes subject to intrinsic and extrinsic signals. Molecular chaperones and co-chaperones, especially heat shock proteins, are ubiquitous molecules involved in the modulation of protein conformational and complexation states. Stem cells exhibit increased stress tolerance and concomitant high levels of chaperone expression [46].

Wang observed that overexpression of Hsp20 protected mesenchymal stem cells (MSCs) against cell death triggered by oxidative stress in vitro. The mechanisms contributing to the beneficial effects of Hsp20 were associated with enhanced Akt activation and increased secretion of growth factors (VEGF, FGF-2, and IGF-1) [47].

**From Our Clinical Experience to a New Method for Treatment of DJD**

Drawing on these historical experiences and considering the new biological knowledge about regenerative medicine, we have begun treating DJD or osteoarthritis (OA) with a new approach based on an anatomical joint-restoring method, using a gel-repairer effective as stimulator of innate stem cells.

Similarly to other authors [12], we started by using Pdrn (Placentex Integran®, 5,625 mg by Mastelli Officina Biofarmaceutica) on DJD during the 1980s, but we observed an unsatisfactory response in terms of tissue repairing detectable in clinical, radiological, and ultrasonography studies. In conclusion Placentex Integran® seemed to cause a short stimulation on tissue before it was absorbed: the effect peaks within 3-5 days of the injection and decreases rapidly in the following days. This pharmaco-dynamic property reduces the potential use of the drug for tissue repair, which often
needs a longer biological stimulation to activate the innate reparative mechanisms.

Moreover clinical results had shown that Pdrn was less effective than Amnios (as Amniex®) membranes.

We considered three possible main causes for this evidence: (1) fast diffusion, low Pdrn absorption and weak stimulation over local innate stem cells, (2) lack of surgical trauma, (3) differences in the nature of substances used in the treatment.

So we thought that treating patients with a mixture of blood and Pdrn would reduce the time of absorption of Pdrn in the articulation. In that experiment, the purpose was to prolong the Pdrn stimulus within the damaged articular area. Patients’ own blood was the obvious choice for compatibility and avoidance of side effects.

When we compared the results of the procedures, Pdrn alone to Pdrn plus blood, the latter resulted in the following: (1) improvement in terms of pain and articular motion, evaluated in 68% of the patients as a good outcome of the therapy, and stable after 12 months (vs. 43% with Pdrn alone); (2) the follow-up showed better long-term results in the group with the blood treatment; (3) the average number of infiltration was reduced by 40%; and (4) there were fewer therapeutic failures.

We concluded that blood worked as a mechanical and chemical support; in fact, hemotoma traps Pdrn with a prolonged release effect and above all, cell hemolysis leads to a release of different products that could increase the effectiveness of Pdrn, such as GF, HSP, cytokines, endogenous nucleotides, and nucleosides.

**Reasoning and Innovation**

As mentioned above, we have devised a new biomaterial (gel-repairer) with the following properties:

1. Chemical and physical compatibility
2. Long absorption time
3. Synergistic effect between Pdrn, HSP, and local GF on innate joint stem cells
4. Neither toxicity nor side effects

Finally, we made a jellified mixture preparation with distressed (low and high temperature), processed blood, Pdrn, and a thickening substance, thus obtaining the gel-repairer.

For the gel-repairer mechanism of action, we developed the following hypothesis, coherent with biochemical and *in vitro* research and supported by clinical evidence:

- The gel-repairer allows prolonged, stimulatory action by polydeoxyribonucleotides and heat shock proteins on innate local stem cells, inducing a reparative mechanism. Moreover, the substance is introduced by means of a minimally invasive surgical procedure, which leads to local traumatic necrosis and hypoxia.
- This local, reparative mechanism activates the tissue microenvironment and enhances the performance of the regenerative gel.

- The gel-repairer can act as a scaffold for stimulated stem cells.

The structural repair that our gel effects over tissues may be produced by enhancing fibroblast stimulation and proliferation and inducing the production of elastin and collagen type II, resulting in an increase in flexibility of the articular capsule and ligaments. An increase of compliance of the bursa follows, which leads to a reduction in the intra-articular pressure and, consequently, of pain.

Another gel effect is probably occurring on periosteal tissue and osteoblast cells, inducing proliferation and bone repair. X-rays have occasionally shown an increase in the cartilaginous matrix layer.

**MATERIALS AND METHODS**

From January 2003 until June 2009, we treated 948 patients for DJD, including virtually all medium and large articulations. The focus of this analysis is on two groups of patients: The first group (I) considered for this study was composed of 86 patients older than 80 years of age and affected by DJD of the hip or/and knee. The second group (II) was composed of 90 patients around 50 years old and affected by the same disease, but in this group the causes of DJD were quite different inasmuch as they consisted of post-traumatic, congenital hip dysplasia, and arthritis induced by severe postural defects.

All patients were referred by orthopedic surgeons and rheumatologists with a clinical diagnosis of DJD or OA in very advanced staging and symptoms with greater than 6 months’ duration.

The first group of patients was judged to be of high surgical risk for surgical prosthesis (most were ASA III-IV), and both groups had been nonresponders to current conservative therapies. All patients in both groups had become nonresponders or intolerant to NSAIDs and corticosteroids.

All patients gave informed consent before entering the study.

Patients who underwent corticosteroids therapy over the last month, INR over 3.5 and affected by acute rheumatic diseases were excluded.

**GEL-REPAIRER: PREPARATION AND USE**

All patients of both groups underwent local treatment with gel-repairer. Gel-repairer is prepared with distressed (low and high temperature) processed blood, Pdrn, and a thickening substance [Patent Pending RM2009A000485].

The quantity of gel that was used to treat the joints depended on the volume of articulation and the thickness of subcutaneous fat. The average quantity of gel used in each point of introduction was 95 mg (range, 55–110) for the hip and 42 mg for the knee (range, 35–60).

Generally, the treatment was performed simultaneously on two or three areas of the joint previously evaluated by clinical and radiological assessment. The area needing treatment was injected with a local anesthetic composed of both mepivacaine 2% (2 mL) and naropine 10% (2 mL), 4–5 mL in total. A minimal incision (5 mm) was made in order to
introduce a Kelly forceps and reach subcutaneous tissue in the periarticular space where the gel was placed, and then the wound was sutured.

The treatment was repeated at weekly intervals. Preliminary results were assessed after three procedures. We considered as nonresponders those patients who did not improve after three treatments.

CRITERIA FOR EVALUATING RESULTS

Clinical Assessment

We adopted a subjective patient-completed questionnaire (WOMAC) of the hip and/or knee, and two clinician schedules, filled by the physician, respectively known as Harris Hip score and Knee Society score.

The Western Ontario and McMaster Universities (WOMAC) osteoarthritis index is a disease-specific, self-administered, health status measure. It probes clinically important symptoms in the areas of pain, stiffness, and physical function in patients with osteoarthritis (OA) of the hip and/or knee. The index consists of 24 questions (5 pain, 2 stiffness, and 17 physical function). The WOMAC is a valid, reliable and sensitive instrument for the detection of clinically important changes in health status following a variety of interventions (pharmacologic, surgical, physiotherapy, etc.) [48-52].

The questionnaire was filled out by patients at baseline before treatment and again during the follow up. Individual question responses are assigned a score between 0 (extreme) and 4 (none). Individual question scores are then summed to form a raw score ranging from 0 (worst) to 96 (best). Finally, raw scores are normalized by multiplying each score by 100/96. This produces a reported WOMAC score between 0 (worst) and 100 (best).

The Harris Hip Score (HHS) was created to evaluate patients’ status after hip prosthesis surgery. Questions are grouped into five categories: pain, motility, functional activities, and physical examination. Scores range between 0 (worst) to 100 (best) [53].

The Knee Society score (KSS) is subdivided into a knee score that rates only the knee joint itself and a functional score (KSFs) to assess the patient’s ability to walk and climb stairs. The knee rating system considers the following main joint parameters: pain, stability and range of motion, flexion contracture, extension lag, and misalignment. Thus 100 points will be obtained by a well-aligned knee with no pain, 125 degrees of motion, and negligible anteroposterior and mediolateral instability. A patient’s joint function considers only walking distance, stair climbing, and walking aids. The maximum function score, which is also 100, is obtained by a patient who can walk an unlimited distance and go up and down stairs normally. The form itself is largely self-explanatory: 50 points are allotted for pain, 25 for stability, and 25 for range of motion. Walking ability is expressed in blocks (approximately 100 meters). Stair climbing is considered normal if patient can ascend and descend stairs without holding a railing, and scoring is largely self-explanatory: 50 points are allotted for pain, 25 for stability, and 25 for range of motion [54].

Radiological Assessment

Patients were classified following the Kellgren and Lawrence Scale (K&L) for radiological assessment of DJD. The scale defines four pathological degrees for OA: Grade I: doubtful narrowing of joint space and possible osteophytic lipping. Grade II: definite osteophytes, definite narrowing of joint space. Grade III: moderate multiple osteophytes, definite narrowing of joint space, some sclerosis, and possible deformity of bone contour (pre-ankylisis). Grade IV: large osteophytes, marked narrowing of joint space, severe sclerosis, and deformity of bone contour (anklylosis) [55, 56].

Follow up was performed within 6 to 12 months (short term) and 24 to 48 months (long term), with WOMAC, Harris Hip score, and/or Knee Society score questionnaires, and the K&L Scale for Radiological Assessment.

Data were expressed as mean ± SD, and statistical analysis was performed using Student’s t test. Differences were considered significant at the level of P < 0.01. Statistical analysis was performed by using GraphPad Instat software (GraphPad Software, Inc; San Diego, CA, USA).

RESULTS

From January 2003 to June 2009, we treated two groups of patients affected by DJD of the hip and/or knee. The first group (I) consisted of 86 patients, of which 49 patients were female and 37 male. The average age was 83 (range, 80–91) (Table 1). The total number of joints treated was 123 (63 knees and 60 hips). Follow-up included 74 patients and ranged from 6 to 48 months. Long-term follow-up included 43 patients (24–48 months), with 48 months as the cut off. Only 12 patients were lost to follow-up (Table 2). The total number of therapeutic procedures performed on the 86 patients was 943 and percentage responding to the therapeutic procedure was 92%. Thirteen patients were treated at different times on the same joint, and 33 patients were treated on more than one joint. In this last case, the results had been analyzed separately. Thirty-four patients underwent physiotherapy.

The second group (II) consisted of 90 patients, of which 51 were female and 39 male. The average age was 51 (range, 45–55) (Table 1). The total number of joints treated was 93 (44 knees and 49 hips). Follow-up included 80 patients and ranged from 6 to 48 months; 52 arrived at the long-term (24–48 months) cut off. Ten patients were lost during follow-up (Table 2). The total number of therapeutic procedures performed on the 90 patients was 800, and we obtained a 91% response to the therapeutic procedure. Eleven patients were treated at different times on the same joint, and three patients were treated at more than one joint. In this last case, the results were analyzed separately.

Sixty-three patients underwent physiotherapy; 21 patients underwent knee arthroscopy before being treated; and eight patients required a prosthesis during the treatment or at the time of the next follow-up. The only complications we found were subcutaneous wound infections on 1.2% of all patients.

The results of the questionnaires are expressed as mean values for basic, short, and long-term assessments as shown in Table 3. The results of the radiological assessments, expressed in the K&L scale for basic, short, and long-term follow-up are reported in Table 4 and (Figs. 1, 2, 3, 4, 5, and 6).
Table 1. Patients’ Clinical Features

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>83</td>
<td>51</td>
</tr>
<tr>
<td>Males/Females</td>
<td>37/49</td>
<td>39/51</td>
</tr>
<tr>
<td>Joint treated</td>
<td>hip/knee</td>
<td>hip/knee</td>
</tr>
</tbody>
</table>

* Patients (N= 86); Lost (N=12).
* Patients (N= 90); Lost (N=10)

NOTE: Lost patients either died during the treatment, did not respond to the treatment, or they were lost to follow up.

Table 2.  Lost Patients

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not responsive</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Lost to follow up</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Died during treatment</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Patients (n= 86); Lost (n=12).
* Patients (n= 90); Lost (n=10).

Table 3. Average Clinical Baseline Assessment and Classification Before and after Treatment

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classifications</td>
<td>Average basic score a</td>
<td>Average short-term score b</td>
</tr>
<tr>
<td>WOMAC</td>
<td>31,3</td>
<td>75,2</td>
</tr>
<tr>
<td>Harris Hip</td>
<td>22,5</td>
<td>64,4</td>
</tr>
<tr>
<td>Knee Society</td>
<td>35,4</td>
<td>76</td>
</tr>
<tr>
<td>Knee Society Function</td>
<td>25,1</td>
<td>54,4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classifications</td>
<td>Average basic score a</td>
<td>Average short-term score b</td>
</tr>
<tr>
<td>WOMAC</td>
<td>31</td>
<td>78,5</td>
</tr>
<tr>
<td>Harris Hip</td>
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<td>67</td>
</tr>
<tr>
<td>Knee Society</td>
<td>36</td>
<td>76</td>
</tr>
<tr>
<td>Knee Society Function</td>
<td>25</td>
<td>60</td>
</tr>
</tbody>
</table>

a Basic score: before treatment.
b Short-term score: within 6 to 12 months of treatment.
c Long-term score: within 24 to 48 months of treatment.
Table 4. Kellgren and Lawrence Scale (K&L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Basic score</th>
<th>Short-term score</th>
<th>Long-term score</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>GIII</td>
<td>GII</td>
<td>GII</td>
</tr>
<tr>
<td>54</td>
<td>7</td>
<td>4</td>
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<td>32</td>
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<td>II</td>
<td>GIII</td>
<td>GII</td>
<td>GII</td>
</tr>
<tr>
<td>50</td>
<td>21</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

*aBasic score: before treatment.
*bShort-term score: within 6 to 12 months of treatment.
*cLong-term score: within 24 to 48 months of treatment.

Fig. (1). Right hip: (A) Grade IV K&L before treatment; (B) Grade III K&L after treatment (6 month follow-up).

Fig. (2). Left Knee: (A) Grade III K&L before treatment; (B) Grade II K&L after treatment (20 month follow-up).

Fig. (3). Right hip: (A) Grade IV K&L before treatment; (B) Grade III K&L after treatment (43 month follow-up).
Fig. (4). Right Knee, severe chondrocalcinosis in DJD: (A) before treatment; (B) after treatment (6-month follow-up). Mainly, the medial compartment of the knee had been treated.

Fig. (5). Left Knee: (A) Grade III K&L before treatment; (B) Grade II K&L after treatment (7-month follow-up).

Fig. (6). Right Knee: (A) Grade III K&L before treatment; (B) Grade III K&L after treatment (8-month follow-up). Clinical improvement was more evident than radiological results.
Results of WOMAC questionnaire in both groups, respectively, for the hip and the knee, show that in the short term after treatment, a clear clinical improvement in terms of perceived pain both at rest and under stress; performance in terms of joint mobility and stability also improved. These results are confirmed in the long term as well (Figs. 7, 8).

The Harris Hip score and the Knee Society function score, which provide objective criteria for the clinical state of the patient, show substantial improvements consistent with WOMAC over the short and the long-term for groups I and II (Figs. 9, 10, 11). In particular, with the Knee Society function score, which evaluates the functional performance of the knee, we may notice that this parameter improves at the same rate as the clinical objective exam (Fig. 11).

Patients of both groups that had a radiological down-staging from G IV to G III according to the K&L scale show an average WOMAC score in the follow-up that is higher compared to the patients with down-staging from G III to G II and, mainly, also compared to the subgroup of patients whose K&L staging remained unchanged. This evidence is statistically significant in group I between stages G IV/GIII and in the subgroup of patients with unchanged K&L scoring (Fig. 12).

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**WOMAC Hip score**

![Graph](image1)

**WOMAC Knee score**

![Graph](image2)
Fig. (9). Harris Hip score before and after treatment at short and long term. Data were expressed as mean ± SD. Differences were considered significant at the level of p<0.01.

□ HH-bs = Harris Hip basic score
■ HHs = Harris Hip score (short / long term)

Fig. (10). Knee Society score before and after treatment at short- and long-term follow-up visits. Data were expressed as mean ± SD. Differences were considered significant at the level of p<0.01.

□ KS-bs = Knee Society basic score
■ KSs = Knee Society score (short / long term)
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**Knee Society function score**

**Group I**

\[ p < 0.001 \]

**Group II**

\[ p < 0.001 \]

**Fig. (11).** Knee Society function score before and after treatment at short- and long-term follow-up visits. Data were expressed as mean ± SD. Differences were considered significant at the level of \( p < 0.01 \).

\( \square \) KS-fbs = Knee Society function basic score

\( \blacksquare \) KS fs = Knee Society function score (short / long term)

**WOMAC and K&L scale correlation**

**Group I**

\[ p < 0.05 \]

**Group II**

**Fig. (12).** Correlation WOMAC classification versus Kellgren and Lawrence scale. Columns represent the average ratio Ws/W-bs in respect to both downstaging and maintenance of K&L scale. Data were expressed as mean ± SD. Differences were considered significant at the level of \( p < 0.05 \).
DISCUSSION

Our clinical experience using nonviable amnios and Pdron over DJD has led to the development of a new substance effective at stimulating innate articular stem-cells in the attempt to regenerate consumed tissue. We tried to create a long-lasting regenerative microenvironment over local stem cells formed by a surgical pocket rich in necrosis products and deficient in oxygen. These conditions are useful for activating local reparative mechanisms through local GFs and other biochemical activators (VEGF, FGF 1-2, TGF-beta1, HIF-alpha, PDGF). The surgical pocket was filled with gel-repairer that is a concentrated mixture of denatured proteins (HSP) and Pdron. This gel matrix, through its active components, could link and gather TGF-beta 1 and other GFs already in the body, rendering useless inoculation of expensive synthetic growth factors.

For this data, two groups of patients were considered. The first group (I) consisted of patients over 80 years old who were judged to be at high surgical risk, and a second group (II) of young (45–55 years old) patients. Both groups were untreatable with standard therapy. All patients were treated with local periarticular insertions of gel-repairer.

The data gathering involved assessment by questioners, which helped to keep track of how the patient felt about the treated joint and how well he/she was able to perform usual activities in terms of pain and function for everyday life. Statistical analysis of results shows a significant improvement of pain and joint motility (Tab. 3). These improvements were similar in both groups and quite stable over time (Figs.: 7, 8, 9, 10, 11, 12).

Radiological modifications were measured using the Kellgren and Lawrence scale. In group (I) 30 out of 74 patients (41%) demonstrated a changed K&L grade in the direction of a down-staging of the disease: 7 patients went from grade III to II, and 23 went from IV to III (Table 4). In group (II) 41 out of 80 patients (51%) showed a down-staging of DJD: 21 went from grade III to II, and 20 went from IV to III (Table 4). These results, though encouraging, only show part of the story. It is impossible to catch all the minute modifications that have great biological and clinical meaning through traditional radiological analysis, given that most of the impact occurs on the fibro-cartilaginous compartment and the capsular-ligament tissue. Patients who improved their K&L grade, however, seemed to show the best clinical results during long-term follow-up. Comparing WOMAC and K&L scale results (Fig. 12), we can observe an evident correlation between structural and clinical improvements. In addition, patients of both groups with greater anatomic improvement have more stable clinical results in the long term.

The question is: What is really causing the clinical and radiological improvements in both patient groups?

It seems that results in terms of reduction of pain and improved articular functionality arise from a relatively stable improvement of the articular biomechanics induced by the gel on the treated areas. The hypothesis is that a prolonged action of proliferative and differentiative stimulus of polydeoxyribonucleotides, Hsp, and of other growth factors on the cellular blastic periarticular compartment, together with the scaffold function that the gel might have on the activated stem cells, produces a structural change in the joint. This is confirmed by the improved articular flexibility that accompanies the reduced pain. This flexibility seems to be caused by a tissue modification that translates into an increased compliance of the ligament bursa tissue, which reduces intra-articular pressure.

Traditional radiology shows an increase in thickness of the soft tissue layer in 30 out of 74 cases and in 51 out of 80, respectively, in the first and in the second group. These patients, in turn, show better overall results in the long term. In other cases, MR shows that the intensity of the signal of the neo matrix is similar to that produced by the fibro-cartilaginous interarticular layer. Furthermore, traditional radiology has limits in its ability to highlight the structural modifications of soft tissue that become apparent when Rx and MR images of the same patient are compared. We believe that the greater specificity and sensitivity of MR will increase the percentage of patients showing a down-staging, once standard criteria are introduced (Fig 13). Given the absence to date of a standard criteria for MR, this data could not be statistically sampled and used in this study but only presented as part of the case report.

The histological interpretation of this radiological and structural joint improvement, expressed as an increase in the thickness of the radiolucent layer between the head joints, still has to be accounted for. We will be investigating this question in a future experimental study.

CONCLUSION

This study suggests a new approach to treatment of DJD based on innate local stem cell stimulation aimed at tissue restoration.

Currently, the treatment of DJD is limited to the following therapeutic options:

(i) NSAID’s, steroids and analgesics;
(ii) Local treatment such as ultrasound, laser, electrophoresis;
(iii) Physiokinesis therapy;
(iv) Surgery (arthroscopy, prosthesis).

Surgical treatment has been the gold standard for advanced degenerative disease.

The advantages of the outlined procedure are:

• minimally invasive;
• patient acceptance and reliance on the procedure;
• the therapy does not preclude additional therapies;
• simple procedural method, applicable on almost all articular areas;
• cost effectiveness;
• no collateral effects, no toxicity, no significant complications.

Our procedure is a new and still developing proposal for the treatment of DJD at any stage of disease, from chronic
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inflammation to degeneration and ankylosis. If further analyses confirm our findings, we may be on the verge of a therapeutic method that will improve patients’ quality of life in terms of joint functionality and pain reduction while saving millions at a health system level. Given that this procedure does not call for any investments and could be performed by most physicians with minimal training, it could spread rapidly and be made available to an ever-increasing aging population.

CONFLICT OF INTEREST

The authors declare that they have no financial or non-financial competing interests.

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PATIENT’S CONSENT

Declared none.

ABBREVIATIONS

ASA = American Society of Anesthesiologists
Classification of Preoperative Risk
DJD = Degenerative Joint Disease
HH-bs = Harris Hip basic score
HHs = Harris Hip score (short / long term)
KS-bs = Knee Society basic score
KSs = Knee Society score (short / long term)
KS-fbs = Knee Society function basic score
KSfs = Knee Society function score (short / long term)

Fig (13). Rx vs MR of a 53-year-old patient before and after treatment. This comparative figure shows the right knee of a man who underwent gel-repairer treatment mostly focused on the medial joint compartment. X-ray imaging results do not change from 2005 to 2010, and so the K&L scale is steady. Comparing MR imagery after and before treatment, however, shows evidence of the recovery of thickness in the soft tissue layer and a great improvement over bone damage through diminishing erosion and pseudo-cyst marrow.
K&L = Kellgren and Lawrence Scale
MR = Magnetic Resonance
NSAIDs = Nonsteroidal anti-inflammatory drugs
OA = osteoarthritis
Pdrn = Polydeoxyribonucleotides
pts = patients
WOMAC = Western Ontario and McMaster Universities
W-bs = WOMAC basic score
Ws = WOMAC score (short / long term)

REFERENCES

[1] Parolini O, Soncini M. Human Placenta: a source of progeni-
[2] Davis JW. Skin transplantation with a review of 550 cases at
[4] Trelford JD, Trelford-Sauder M. The amnion in surgery, past and
venous leg ulcers with prepared human amnion. Br J Plast Surg
Psychiatry 1999; 156: 630.
[10] Vishwakarma GK, Kahare AK. Anmiotic arthropathy for tubercul-
data on polydeoxyribonucleotides of human placenta. Int J of Tis-
e degli estratti placentari sulla sintomatologia algica dell’artrosi.
Ortopedie e Traumatologia Oggi 1987; 7: 47-56.
[13] Born GV, Kratzer MA. Source and concentration of extracellular
adenosine triphosphate during haemostasis in rats, rabbits and man.
[14] Ding GJ, Ning Na W, Heppel H. Extracellular ATP shows Syner-
gistic enhancement of DNA synthesis when combined with agents
that are active in wound healing or as neurotransmitters. Biochem
purine nucleosides stimulate cell division and morphogenesis:
pathological and physiological implications. Med Hypotheses
of cell division and differentiation: mechanism and pharmaco-
[18] Chavan AJ, Huley BE. Interaction of nucleotides with acid fibro-
[19] Bourque WT, Gross M, Hall BK. Expression of four growth-
[20] Bower W, Buckley KA, Garland A, Hipkind RA, Bilge B,
Gallagher JA. Extracellular Nucleotide signalling: a mechanism for
integrating local and systemic responses in the activation of bone
[21] Canalis E, Mc Carthy T, Centerella M. Growth factors and the
[22] Joyce ME, Jingushi S, Bolander ME. Tranforming growth factor
[23] Joyce ME, Jingushi S, Scully SP, Bolander ME. Role of growth
factors in the fracture healing. Prog Clin Biol Res 1991; 365: 391-
416.
[24] Henning UGG, Wang Q, Gee NH, Von Borstel RC. Protection and
repair of g-radiation induced. lesions in mice with DNA or Deox-
Polydeoxyribonucleotides enhance the proliferation of human skin
fibroblasts: involvement of A2 purinergic receptors subtypes. Life
Sciences 1999; 64: 1661-74.
of polydeoxyribonucleotides on human fibroblasts in primari
[27] Nakamura E, Uezono Y, Narusawa K et al. ATP activates DNA
synthesis by acting on P2X receptors in human osteoblasts like
[28] Vega C, Desai A, Montesinos MC, Cronstein BN. Adenosine A2a
receptor agonists promote more rapid wound healing than recombi-
nant human platelet derived growth factor (Beclaplermin Gel). J In-
[29] Edington SM. DNA and RNA therapeutics: unsolved riddles. Bio
[30] Adjei AA, Yamamoto S, Kulkarni A. Nucleic acid and/or their
components: a possible role in immune function. J Nutr Sci Vita-
minol 1995; 41: 1-16.
[31] Lane JM, Tomin E, Bostrom MPG. Biosynthetic bone grafting.
(PDRN) promotes human osteoblast proliferation: A new proposal
[33] Guizzardi S, Martini D, Bachelli B et al. Effects of heat depro-
trinate bone and polynucleotides on bone regeneration: An experi-
[34] Abdollahi H, Harris LJ, Zhang P et al. The Role of Hypoxia in
Stem Cell Differentiation and Therapeutics. J Surg Res 2011; 165:
112-7
[35] Nakai T, Mochida J, Sakai D. Synergistic role of e-MyC and
ERK1/2 in the mitogenic response to TGF beta-1 in cultured rat
growth factor 2 increases the multipotentiality of human adipose-
derived mesenchymal stem cells. Stem Cells 2008; 26(6): 1598-
608.
et al. FM19G11, a new hypoxia-inducible factor (HIF) modulator, af-
facts stem cell differentiation status. J Biol Chem 2010; 285:
1333-42.
[38] De Maio A. Heat shock proteins: facts, thoughts, and dreams.
[39] Wu C. Heat shock transcription factors: structure and regulatio-
[40] Santoro MG. Heat shock factors and the control of the stress re-
2004; Appendix J: Appendix 1T.
[42] Raboy B, Sharon G, Parag HA, Shochat Y, Kulka RG. Effect of
stress on protein degradation: role of the ubiquitin system. Acta
[43] Walter S, Buchner J. Molecular chaperones-cellular machines for
[44] Borges JC, Ramos CH. Protein folding assisted by chaperones.
[45] Nishikawa M, Takemoto S, Takakura Y. Heat shock protein deriva-
tives for delivery of antigens to antigen presenting cells. Int J
[46] Prinsloo E, Setati MM, Longshaw VM, Blatch GL. Chaperoning
stem cells: a role for heat shock proteins in the modulation of stem
stem cells are resistant to oxidative stress via enhanced activation
of Akt and increased secretion of growth factors. Stem Cells 2009;
[48] Bellamy N. WOMAC: a 20-year experiential review of a patient-
centered self-reported health status questionnaire. J Rheumatol
Self-Repair in Degenerative Joint Disease


