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Opinion Article

Technology rationale for the development of CellularMatrix® A-CP-HA Kit, certified medical device allowing the combination of platelet rich plasma and hyaluronic acid for the treatment of osteoarthritis

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The composition of synovial fluid of patients suffering from osteoarthritis (OA) is altered with reduced concentration and molecular weight of Hyaluronic Acid (HA) and augmentation of catabolic enzymes and inflammatory markers. Intra-Articular (IA) injections of exogenous HA aim to restore the rheological properties of the synovial fluid in the osteoarthritic joints. However, clinical studies have shown that the benefit of HA injections lasts only for around 6 months and that many patients don't respond well to repeated course of HA treatment. On the other side, platelet rich plasma (PRP) IA injections have been shown to reduce pain and improve joint mobility, probably by modulating the expression of catabolic enzymes and inflammatory markers. As injections of HA and PRP use different pathway to alleviate symptoms in OA patients, the concept of combining these two treatments has emerged recently. *In vitro* evaluations and preliminary studies have demonstrated the therapeutic potential of this new therapeutic approach for OA treatment. To meet the needs of medical practitioners that want to treat their patients with this new treatment option, an innovative medical device, the CellularMatrix A-CP-HA Kit, has been specifically designed. This device is the first certified medical device that allows the combination of PRP and HA in manner compliant with medical devices regulation and good clinical practice.

Keywords: CellularMatrix • A-CP-HA Kit • platelet rich plasma • hyaluronic acid • knee osteoarthritis • regenerative medicine

Introduction

Osteoarthritis is a degenerative disease and represents by far the most common cause of articular pain [1]. It is associated with degeneration of the joint, subchondral sclerosis and inflammation of the synovial membrane [2]. The evolution of this condition is characterized by the following symptoms: pain, joint cracking/popping, stiffness, deformity, loss of mobility and especially in the case of knee OA, swelling and synovial effusion. The disease is related to aging and generally affects the joints that are subject to stress, such as the knee, hips, small joints of the hand, and the cervical and lumbar spine [2]. OA is the most common joint disorder in the United States. Among adults 60 years of age or older, the prevalence of symptomatic knee OA is approximately 10% in men and 13% in women. The number of people affected with symptomatic OA is likely to increase due to the aging of the population and the obesity epidemic [3].

In a healthy joint, cartilage and synovial fluid allow for shock absorption and for the bones to slide over one another with ease, thus ensuring the joint mobility. Synovial fluid, that fills the intra-articular join space, lubricates the joint and is a source of nutrients for the articular cartilage. It is mainly composed of HA, a highly hydrophilic molecule that gives to the synovial fluid its shock-absorbing properties. It has been shown that the HA concentration and molecular weight decrease in synovial fluid with age and in patients suffering from OA, which may cause symptoms of pain and physical loss of function. Treatment with IA injections of exogenous HA aims to restore the shock absorbing and lubrication properties of the synovial fluid in the osteoarthritic joints [4].

The synovial fluid of patients suffering from OA also contain increased catabolic and inflammatory markers [5]. PRP IA injections have been shown to decrease pain and improve joint mobility [6], but also to regulate the expression of catabolic and inflammatory markers in synovial fluid [7,8].

As injections of HA and PRP use different pathway to alleviate symptoms in OA patients, the concept of combining these two treatments has emerged recently. *In vitro* evaluations and preliminary studies have demonstrated the therapeutic potential of this promising new therapeutic approach for OA treatment. The CellularMatrix A-CP-HA Kit has been specifically designed, and certified, for allowing the combination of PRP and HA in a manner compliant with medical device regulations and good clinical practice. The device is a closed-circuit system in which autologous PRP is prepared from a small volume of the patient's blood in presence of a HA solution that is preloaded inside the device. The resulting product is a leukocyte poor PRP resuspended in a three-dimensional matrix of HA. It combines the mechanical properties of HA with the biological properties of PRP that act synergistically to alleviate the OA symptoms.

Hyaluronic acid in the treatment of osteoarthritis

HA is a naturally occurring linear polysaccharide, widely distributed in human tissues, where it constitutes the major part of the extracellular matrix. HA is a major component of synovial fluid and it is known that its concentration and its molecular weight are lower in synovial fluids from patients suffering from OA. Visco-supplementation treatment by intra-articular injections of exogenous hyaluronic acid solution is currently one of the possible treatment for OA, especially of the knee. HA IA injections are designed to improve HA content in synovial fluid and restore its rheological properties and its mechanical action on the cartilaginous structures of the joints, leading to a reduction of pain and an improvement of joint function. It has been demonstrated that locally injected HA is rapidly degraded, so it is thought that the long-term clinical benefits of HA may be due to its ability to promote the *de novo* synthesis of a high molecular weight HA, rather than just replacing the degraded endogenous HA.

In vitro, *in vivo* and clinical studies demonstrate that exogenous HA may also mediate therapeutic effects in OA by many other biochemical actions within the joint, including induction of proteoglycan aggregation and proteoglycan synthesis, inhibition of inflammatory mediators, and analgesic activity [9].

For almost two decades, chemically modified HA derivatives have been developed to increase their molecular weight and thus their residence time within the joint. The resulting so-called hylans, made of chemically cross-linked HA molecules, exhibit a higher viscosity and consequently higher half-life in the joint, suggesting a potentially better effectiveness. Whether hylans do really show a greater efficacy is still controversial, as available data is not sufficient to be able to draw reliable conclusions [10,11].

The therapeutic effects of IA HA injections may last for a relatively long period, ranging from several weeks to several months [12-23]. In 2011, a systematic review showed evidence of a modest but significant efficacy of intra-articular HA for knee OA compared to a placebo four weeks post-injection with a moderate clinical significance after eight weeks and continued residual benefit until 6 months post injection [24].

Nevertheless, some meta-analyses [25,26] highlighted the fact that the extent of these positive effects appears to be only modest form the clinical point of view. Indeed, the authors showed that even though the outcome parameters exhibited statistically significant differences in favor of HA over the saline placebo, pain relief and functional improvement do not seem to represent an important clinical benefit. One reason may be that there was a generally high placebo effect. Possible explanations for it include synovium aspiration (arthrocentesis), as well as the injections per se, which are known to provide beneficial effects by themselves, making it difficult to distinguish them from the beneficial effects of the HA. In addition, HA has been shown to have a high rate of non-responding patients, 50% to 20% of the patients, depending on the studies and the HA used [27-31].

IA injections of HA represent an efficient and safe approach for the treatment of OA, particularly if it is considered within the framework of a global treatment, which also includes medication-based and non-medication-based therapeutic interventions, such as physical exercise and weight loss. Furthermore, because HA injections have no known medication interactions, it is a good option for patients on multiple medications [32].

A recently conducted literature search on a HA (Ostenil®, TRB Chemedica, Switzerland), with similar characteristics to the one contained in CellularMatrix A-CP-HA Kit, identified 12 studies [33-44]. In total, 694 patients suffering

from various degenerative joints conditions, including knee OA (62%), hip OA (17.5%), hallux rigidus (5.5%), temporomandibular joint disorders (7%) and rhizarthrosis (8%) were treated with Ostenil® or Ostenil®Mini according to different treatment courses (one to five intra-articular injections). All studies, except two case reports on acute pseudoseptic arthritis, showed that pain relief could be obtained after the first intra-articular injection, and was continuously improved with an increased number of injections. Additionally, functional improvement could also be obtained. Generally, HA effects could last for up to 6 months. In all studies, the product proved to be safe and well-tolerated, as no serious adverse reactions were observed.

Platelet rich plasma in the treatment of osteoarthritis

PRP is a volume of autologous plasma that has a platelet concentration above the baseline value in whole blood, [45]. As such, PRP contains not only a high concentration of platelets but also the full content of plasma clotting factors, the latter of which typically remain at their physiologic levels [46]. The rationale of PRP use for therapeutic applications is to mimic the biological healing process that normally occurs in the human body after injury [47]. PRP preparation consists in removing red and white blood cells, which delay the healing and concentrating platelets, thereby increasing factors that are useful in healing [48]. Because there are numerous PRP preparation protocols, differing by preparation devices, centrifugation conditions and operator dexterity, PRP is used to qualify biological products that greatly vary in their platelet concentration, quality and content in growth factors, and level of contamination with red blood cells and pro-inflammatory white blood cells [49].

This large variability in PRP preparations creates a challenge when trying to accurately draw conclusions from the literature to guide PRP production and determine indications for use. To fulfill the need of a standardized PRP preparation, Regen Lab SA, Switzerland, has developed a PRP preparation technology that works in closed-circuit, using polymer separating gels in evacuated tubes. Blood components are separated according to their specific density thanks to centrifugal force and the separating gel inserts itself precisely between the platelets and the white blood cells. At the end of the centrifugation, the separating gel forms a physical barrier that efficiently isolates the platelets and the plasma in the upper part of the device while red and white blood cells are entrapped below the separating gel, in the lower part of the device. The resulting PRP is a plasma type PRP, that is a PRP with virtually no red blood cells and a very low level of pro-inflammatory white blood cells. The platelet concentration factor is low (1.6 times higher than baseline in whole blood) as the full volume of plasma is recovered, however it has been demonstrated that plasma type PRPs, also called leukocyte poor PRPs, are therapeutically efficient with a platelet concentration factor between 1 and 3 times over the baseline. The so-called therapeutic platelet concentration of 1 billion per ml (4 to 5 times over the baseline values) [45] concerns only leukocyte-rich PRP, as a higher platelet concentration is needed to compensate for the negative effects of pro-inflammatory white blood cells (neutrophils).

IA injections of autologous PRP, and more specifically of leukocyte poor PRP [50], has been found to be an attractive treatment option for the treatment of OA, due to the biological mechanism of action and the autologous nature of the product. A number of *in vitro* studies have already shown the impact of isolated growth factors on the chondrogenic stimulation and differentiation of Mesenchymal Stromal Cells (MSCs). For example, it was shown that MSCs produce significantly more proteoglycans and type II collagen when cultured in presence of TGF-β [51], while bFGF induces chondrogenic proliferation and differentiation of MSCs [52]. PRP represents a safe and cheaper alternative to recombinant growth factors, releasing an autologous and appropriate cocktail of growth factors within a natural and physiological range at the site of injection. *In vitro,* it has been shown that PRP has a proliferative effect on autologous chondrocytes and mesenchymal stem cells [53,54] and attenuates their pro-inflammatory chemokine and metalloproteinase expression [55]. Clinical studies evaluating the efficacy of PRP, prepared with Regen Lab technology, for the treatment of knee osteoarthritis demonstrated significant effects on pain relief and functional improvement, especially for those patients with lower degrees of OA, with safety of use and no serious adverse events attributable to the treatment [8,56-62].

Combination of PRP and HA prepared with the CellularMatrix A-CP-HA Kit

The combination of PRP and HA injections has been suggested as a promising treatment option for osteoarthritis. *In vitro*, it has been

demonstrated that the combination of HA and PRP can synergistically promote cartilage regeneration and inhibit OA inflammation markers [63]. Studies with consecutive injections of PRP and HA on patients suffering from moderate OA suggested that the combination of the two treatments provided an added benefit compared to each product administered individually [64-66]. The results of a case study showed for each patient an increase in the joint space width through objective X-ray measurements [66]. This suggests that the combination of PRP and HA injections may induce local cartilage regeneration and that this therapy may be an effective ultimate chance for patients whose last option might be a total arthroplasty. However, it is not known how PRP interact with HA when the two products are injected consecutively. In addition, there is no safety assessments for this type of procedure.

HA products intended for IA injections are class III medical devices, according to European classification. This imply that they should be used as indicated in their instructions for use and thus not be modified in any way or combined with any other products by the user. To meet the needs of medical practitioners that want to treat their OA patients with PRP combined with HA, Regen Lab has designed the CellularMatrix A-CP-HA Kit. This kit is a certified class III medical device specifically intended for the preparation of PRP in presence of HA and the injection of this combination in a single procedure. The CellularMatrix technology is the first available on the market that allows this combination and is protected by numerous worldwide patents of Antoine Turzi (US8945537, US9517255, EP2544697B1, EP3184114B, JP6076091, JP6321119, CN103079577B, IL221133, CA2789533C, AU2011225828B, RU2614722, KR20130067247, HK1179507, US2015151858, EP2771241, WO2016083549, WO2013061309, WO2011110948).

The European certification procedure for medical devices guarantees that they me et all regulatory requirements. Pre-clinical tests must be performed to demonstrate, among others, biocompatibility of the device components, stability of the device during its shelf life, maintenance of its sterility. Clinical evaluation must be performed for initial conformity assessment and on an ongoing basis to insure post-market surveillance and clinical follow-up. These requirements ensure the safety and the performance of medical devices for their intended use.

Principle of operations of the CellularMatrix A-CP-HA Kit

The A-CP-HA device is a sterile evacuated tube in which 6 ml of the patient's blood are automatically collected by connecting the tube to a blood collection set. The tube contains 2 ml of hyaluronic acid solution at 20 mg/ml, a biologically inert separating gel and 0.6 ml of anticoagulant (sodium citrate 4% (w/v) solution). This anticoagulant is aimed to anti-coagulate only the volume of blood that is collected in the device. It has no ancillary effect on the patient and its action is fully reversible. As for Regen Lab PRP devices, platelets and plasma are isolated from the other blood components by the separating gel through a simple centrifugation (Figure 1). During the centrifugation, the separating gel migrates in the device and intercalates itself precisely between blood components, while the HA gel migrates from the bottom of the device to the top of the plasma, thanks to its low density. At the end of centrifugation, platelets, plasma and the HA gel are isolated in the upper part of the device, while red and white blood cells are entrapped below the separating gel, that forms a physical barrier in the middle of the device. Upon gentle inversions of the device, platelets are put back in suspension in the plasma and, by using a transfer device, the resulting PRP combined with the HA gel is collected in the syringe that will serve for its injection (Figure 2). The preparation procedure is easy, rapid and done in closed-circuit. Provided that proper aseptic techniques are used during the procedure, this technology allows to avoid microbial contamination of the biological sample and operator exposure to the blood of the patient.

The PRP-HA combination prepared with the CellularMatrix A-CP-HA Kit (CM-PRP-

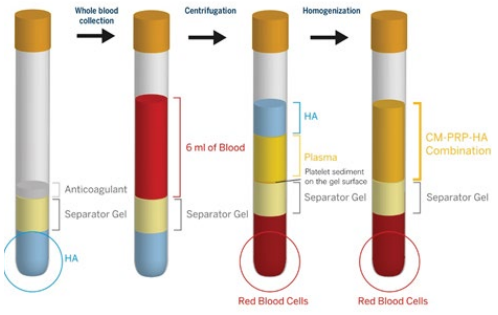

Figure 1. Schematic illustration of the preparation process.

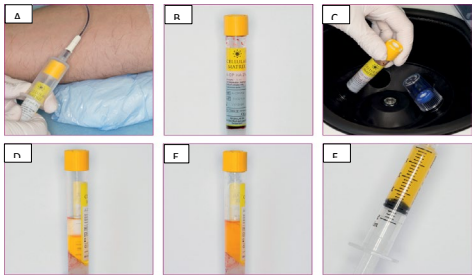

Figure 2. CM-PRP-HA mix preparation process: A: Automatic blood collection inside the A-CP-HA tube; B: Blood filled A-CP-HA tube; C: Centrifugation; D: At the end of the centrifugation the plasma and the platelets are recovered in the upper part of the device. The HA float over the plasma. E: After repeated tube inversion a homogeneous preparation is obtained. F: The resulting CM-PRP-HA mix is ready for injection.

HA) consists of around 3 ml of leukocyte poor PRP, whose composition is known to be therapeutically efficient for the treatment of OA [8,56-62], in suspension in 2 ml of natural HA that form a three-dimensional network. The HA is produced by bacterial fermentation, thus free of animal molecules and not chemically modified. It has similar characteristics to Ostenil® Plus (TRB Chemedica, Switzerland) however without the adjunction of mannitol. When injected into a joint, this HA network not only brings visco-supplementation in the intra-articular space, but also entraps the platelets and the plasma molecules, which probably optimize the biological action of the PRP [67].

Clinical Results

A pilot multicenter study was conducted in France to evaluate the safety and efficacy of the CM-PRP-HA combination obtained with CellularMatrix A-CP-HA Kit in a total of 77 patients suffering from mild to moderate knee OA, who had failed to respond adequately to previous treatment with HA alone [68]. The treatment with CM-PRP-HA (3 injections at day 0, day 60 and day 180) significantly reduced pain at walking between baseline and D270. The percentage of responders according to the criteria of the Outcome Measures in Rheumatology Clinical Trial and Osteoarthritis Research Society International was 94.4%. CM-PRP-HA provided long-lasting benefits for half of the patients and allowed avoiding surgery for almost 80% of them at four years. Another study retrospectively compared patients treated with CM-PRP-HA (40 patients) to a control group treated with PRP only [67]. The treatment was based on 3 intra-articular injections administered at weekly intervals. Clinical results showed a statistically significant improvement compared to baseline over a six-month follow-up period for both groups. This study was not able to show a significant difference between the two treatments probably because the injection interval of one-week for both PRP and CM-PRP-HA was not optimized and too short. Indeed, it has been shown that optimal injection protocol for Regen Lab PRP is with one-month interval [60,61]. A third study compared patients treated with CM-PRP-HA (50 patients) to a control group treated with HA only [69]. The treatment was based on 3 intra-articular injections administered at a three-week interval. Patients were prospectively, clinically evaluated before the treatment and at 2, 6 and 12 months follow-up visits. At each follow up visit, both groups showed a highly significant improvement when compared with the basal assessment. The infra-group comparison showed, at each follow up evaluation, a significantly higher improvement for the group treated with CM-PRP-HA in respect to HA alone.

The side effects observed with CM-PRP-HA were similar to those observed with PRP IA injections. These adverse reactions appeared at the injection site and were mild inflammatory reactions, which were solved in a few days.

Conclusion

The CellularMatrix device, allows to combine PRP with HA in a safe an efficient manner, in respect with medical device regulations and good clinical practice. The clinical results show that the resulting CM-PRP-HA combination brings long term symptomatic amelioration in patients with mild to moderate knee OA (II-III OA grade, according to Kellgren and Lawrence grading scale), even on patients who had failed to respond to previous visco-supplementation with HA alone, and postpone the need for arthroplasty. The HA present in the combination seems to improve the biological action of PRP and thus to bring superior and longer lasting results than standard treatments with PRP or HA alone. Further clinical studies are needed to establish the most efficient treatment protocol (number of injections, interval between injections) for large joints (knee, hip, shoulder) but also for small joints (elbow, wrist, hand, ankle, foot) and for the different grade of OA at these various anatomical locations. The CM-PRP-HA combination seems to be a promising new treatment option and might be an answer for unmet therapeutic needs for patients suffering from OA.

Disclosure of Interest

Solange Vischer, MSc. and Valérie de Fourmestraux, PhD are employees from Regen Lab SA. Antoine Turzi is the CEO of Regen Lab SA.

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