CLINICAL

E. Guidi

THE TREATMENT OF OSTEO-ARTICULAR PATHOLOGIES WITH GUNA COLLAGEN MEDICAL DEVICES

SUMMARY

Collagen is the most abundant structural protein found in the connective, cartilaginous, tendon and bone Tissues of most animals. In humans, its synthesis begins to decline around the age of 50, resulting in cartilage and tendon degeneration and the inevitable development of Arthrosis and Tendinopathies.

These degenerative forms are very frequent and result in symptoms where pain and joint stiffness are the main characteristic.

Conventional therapeutic strategies are based on the use of infiltration with steroid drugs, with the primary objective of relieving patients from pain, without considering the short and long-term negative effect of these treatments.

– This highlights the importance of having therapeutic tools to effectively treat inflammatory and degenerative osteo-articular pathologies, without causing adverse effects, favouring the restoration of Tissues and the *restitutio ad integrum*.

– In this clinical study, 18 patients suffering from joint and tendon disorders frequently encountered in clinical practice were enrolled, all of whom were treated solely by intra- or perilesional administration with Collagen Medical Devices.

The NRS pain scale and ultrasound images were taken into account before and after each treatment, showing significant efficacy with these instruments and their absolute tolerability.

KEY WORDS

COLLAGEN, GUNA COLLAGEN MEDICAL DEVICES, ULTRASOUND-GUIDED AND ULTRASOUND-ASSISTED INJECTIONS, MUSCULO-SKELETAL PATHOLOGIES

INTRODUCTION

THE COLLAGEN

Collagen is the most abundant fibrous protein found in the Animal Kingdom; it is highly represented in connective Tissues and in various organic structures, particularly including tendons, ligaments, joint capsules, cartilage, bones, and skin.

– On the one hand, collagen provides mechanical support to Tissues and, on the other hand, plays an important role in controlling adhesion, cell migration and repair (Randelli *et* Al., 2018).

Understanding its particular structure rationalises the biological functions of this extraordinary hierarchically ordered protein, beginning with the primary structure through to the four elements (Zhao *et* Al., 2021).

– The smallest sub-unit is the tropocollagen, consisting of triplets of glucose/galactose units and 4 amino acids (proline, hydroxyproline, glycine and lysine).

The tropocollagen, organised in a **triple alpha helix dextrorotatory**, gives rise to the mature collagen; the collagen is subsequently structured into fibres, fundamental units that when variously organised participate in the scaffolding of the Tissues and the Extracellular Matrix (ECM).

– Several collagen molecules are bundled together to form a fibril; this joining takes place through a particular mode of anterior 'slipping' in front of each single molecule on top of the other equal

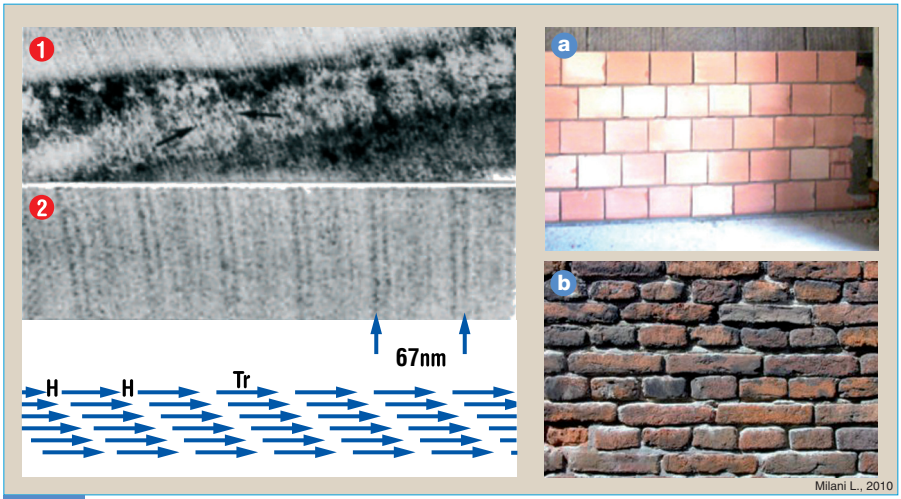


FIG. 1

1: Collagen-bound sugars (ruthenium red colouring)**. Correlation of sugar** (black precipitates) **to the periodicity of collagen fibrils** (ME 112,000X)**;**
2: Section of collagen fibrils (ME 240,000X)**. A cycle of 67 nm** (670 A) **is formed on the basis of collagen molecules slipped by 1/4 of its own length.**
a This positioning of bricks responds well to pressure stresses from above, less so in the case of tangential ones.
b This positioning of bricks responds well both to pressure from above and to tangential forces: in this arrangement, there is a *displacement* of many bricks with respect to those surrounding about ¼ of the length of the individual element.

– Taken from: Milani L. – A new and refined injectable treatment for musculoskeletal disorders – Bioscaffold properties of collagen and its clinical use. PRM, **2010**; 3-15.

to 1/4 of its own length.

The result is therefore a kind of biological wall in which the individual constituent bricks are out of phase with each other in such a way that they produce considerable resistance both against tangential and perpendicular incident forces (Milani, 2010) (FIG. 1).

– In order to be functional, almost all joints must possess two seemingly contrasting fundamental characteristics: stability and mobility.
Joint stabilisation and restraint systems are represented by structures that define the Extra-articular Compartment and the Intra-articular Compartment; collagen is abundantly present in both.

The Extra-articular Compartment consists of ligaments, a joint capsule, tendons and muscles; the Intra-articular Compartment consists of ligaments (only in the knee and hip joints) and articular cartilage.
– In humans, the neosynthesis of collagen begins to decline from **50-60 years** of age; from this age, there is a quantitative depletion and suffering of the articular structures (functional de-resilience).

In particular, with regard to the Locomotor Apparatus, cartilaginous surfaces thin and degenerate leading to Osteoarthritis, while the tendinous and ligamentous structures become less resistant and undergo Tendinosis and Tendinopathies of varying degrees (Ottaviani, 2014).

Tissues in the Locomotor System can be damaged by:
– Wear (overload)
– Traumatic events (which an inflammatory component is always associated to)
– Physiological ageing processes.

In all cases, the most evident aspect of the damage is the loss of the structural integrity to the collagen fibres, which are no longer linearly organised and parallel to each other and may present lacerations of varying degrees (Fung *et* Al., 2010; Milani, 2010).

– The injured Tissue undergoes a long process of multi-phase recovery in which the phenomena of repair and *restitutio ad integrum* are linked to the deposition and reorganisation of the ECM scaffold, consisting mainly of collagen.

Therapy with anti-inflammatory drugs (ASA, NSAIDs) is only useful in the first hours after the traumatic injury; the prolonged use of anti-inflammatory drugs during the repair and remodelling phases may prove detrimental because it negatively interferes with the synthesis of new collagen (Christensen *et* Al., 2011).
– Tissue repair following damage is a complex metabolic process; the end result depends solely on the regenerative capacity of the tissue and the quality of the inflammatory response.

Inflammatory cells at the site of the wound produce cytokines, metabolites and growth factors.
If the response goes from being well 'orchestrated' to being dysregulated, the inevitable outcome will be that of a progressive Fibrosis (defined as an aberrant accumulation of connective Tissue) that will tend to become chronic, causing a loss of function to the affected Tissue (Eming *et* Al., 2017).

– This clinical study examined different pathologies found in the personal musculoskeletal ultrasound study.

The proposed treatment was carried out through ultrasound-guided or ultrasound-assisted injections with some Guna Collagen Medical Devices (see below).

GUNA COLLAGEN MEDICAL DEVICES

Guna Collagen Medical Devices provide collagen in the form of tropocolla-

gen; there is therefore no pharmacological action as there is no modification of the normal metabolism/catabolism of collagen.
– The tropocollagen present in Guna Collagen Medical Devices therefore acts as a bio-scaffold.

– Anisotropy, a fundamental property of well-structured collagen (Wenger *et* Al., 2007), is progressively lost when collagen fibres are damaged by ageing, wear and tear or a traumatic event (Friedrichs *et* Al., 2007; Fung *et* Al., 2010).

Restoring the anisotropy of damaged collagen fibres by injection *in situ* of Guna Collagen Medical Devices is equivalent to inducing the same biological response that is obtained with the eccentric work, characteristic of the phases of functional recovery after a tendon injury and therefore, through the signaling produced by the stimulation of the integrins, induce the cascade of Growth Factors [TGF-β 1 (Trophic Growth Factor β 1), CTGF (Connettive Tissue Growth Factor), IGF-1 (Insulin-like Growth Factor-1)] necessary for the production of new collagen by the fibroblast (Silbernagel *et* Al., 2011).

This is a sophisticated mechanism that, through the local injection of collagen, reactivates the fibroblast's ability to synthesise new collagen, inducing the autologous repair and remodelling mechanisms in the altered connective Tissue. Furthermore, fibroblasts are capable of generating tensile forces, as well as receiving them; these contraction forces of the fibroblast are indispensable for the processes of healing wounds.

– The collagen contained in Guna Collagen Medical Devices is type I porcine collagen; this ensures high levels of safety due to its virtual absence of immunogenicity, making it the material of choice for numerous applications (Brandao *et* Al., 2013).

It is injected locally where it is needed in order to **replace, strengthen, structure** and **protect** Tissues, improving the anatomical and functional structure of the structures containing it, whilst providing mechanical support to the areas involved.

– Guna Collagen Medical Devices have different formulations that make them unique, highly specific and indicated for various Anatomical Districts; the particular tropism is guaranteed by the presence of ancillary substances associated with collagen.

The Guna Collagen Medical Devices used in this study are:

- **MD-Shoulder**, which contains the ancillary Iris, traditionally used to treat joint pain; in particular the stinging pain in the shoulder joints (Rahman *et* Al., 2003; Schutz *et* Al., 2011).
- **MD-Neural**, which contains the ancillary Colocynthis, effective in alleviating nerve-derived pain and articular pain (Nawash *et* Al., 2013; Hussain *et* Al., 2014).
- **MD-Knee**, which contains the ancillary Arnica, to counteract inflammation, especially traumatic inflammation, which frequently affects the knee.
- **MD-Tissue**, which contains the ancillaries Ascorbic Acid, Magnesium Gluconate, Pyridoxine Hydrochloride, Riboflavin and Thiamine Hydrochloride.

– Ascorbic Acid (Vitamin C) is one of the most effective biological hydro-soluble antioxidants, capable of neutralising many reactive Oxygen and Nitrogen species (Wilson, 2009; Masaki, 2010).
– Magnesium Gluconate is one of the most important Magnesium carriers, the deficiency of which can lead to the on-

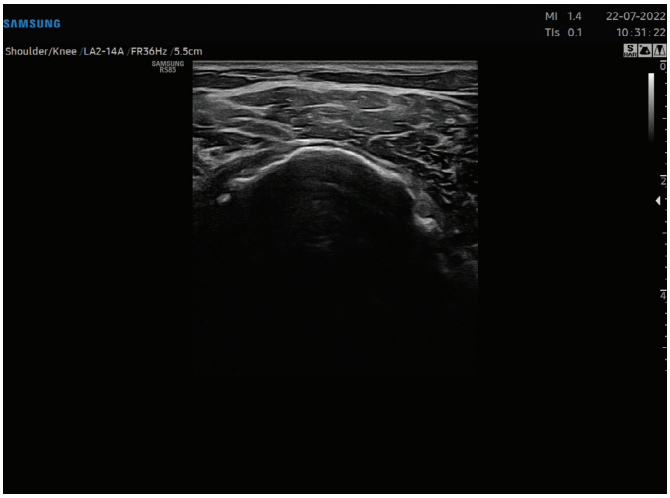


FIG. 2
Subacromion deltoid Bursitis and rupture of the supraspinatus tendon.

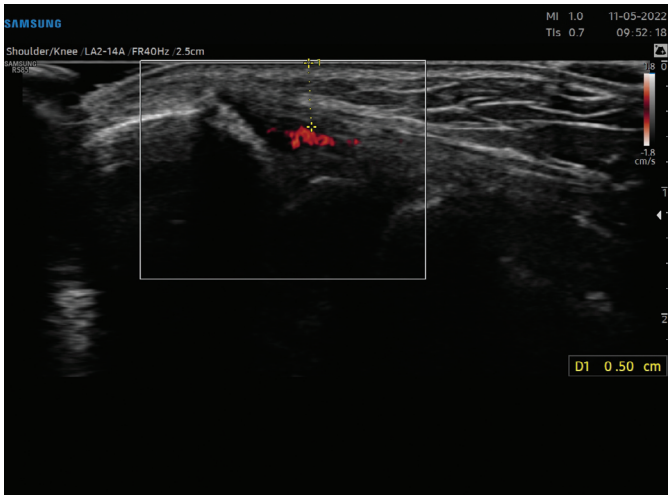


FIG. 3
Epicondylitis. Hypervascularised Tissue.

set of many clinical pictures, including Osteoporosis.
– Pyridoxine (Vitamin B6) has anti-neurotoxic activity (Ngamphaiboon *et* Al., 2010).
– Riboflavin (Vitamin B2) has antioxidant activity (Keil *et* Al., 2013).
– Thiamine Hydrochloride (Vitamin B1) has an antioxidant and detoxifying activity.
The combination of these substances with collagen creates a defence barrier against free radicals and counteracts the ageing of connective Tissue.

MATERIALS AND METHODS

In this study, **18 patients** aged between 26 and 76 years old (mean age 52 years old), 6 M and 12 F, who presented different osteo-articular and musculoskeletal pathologies (TAB. 1):
– Subacromion deltoid Bursitis (6 patients)
– Peritrochanteric Bursitis (4 patients)
– Epicondylitis (2 patients)
– Carpal tunnel Syndrome (1 patient)
– De Quervain's Syndrome (1 patient)
– Achilles Tendonitis (1 patient)
– Hammer biceps insertional Tendonitis on radius (1 patient)
– Post-surgical scar with pericatricial algic nodules (1 patient)
– Chondropathy and Gonarthrosis (1 patient).

All patients underwent an **ultrasound** with the Samsung Rs85 Prestige ultrasound and 14-18 MHz linear probe.

All patients were asked to indicate the degree of pain through the administration of the **NRS scale** (Editor's note: Numerical Rating Scale; 10 = maximum possible pain; 0 = absence of pain).

– In the ultrasound, injured Tissues were also studied with an **elastosonography** to assess the degree of rigidity of damaged structures and with the **MV Flow colour module**, capable of identifying even very small vascular flows.

In cases of Subacromion deltoid Bursitis, the ultrasound revealed a thickening of the bursa walls; in cases of associated rupture or tendon injury of the rotator cuff (FIG. 2) also present was the delamination of the sleeve on the long head of the humeral biceps muscle.
In some cases, joint effusion was also present.

On the colour Doppler, in all patients pain corresponded to increased vascularisation at the site of the Tissue damage; in the case of tendons and ligaments, there was also alteration of the elastosonography, indicative of loss of the collagen fibrillar structure.
– This, if left untreated, not only causes pain, but also exposes the Tissue to a high probability of rupture.

At each session, an ultrasound was performed to assess the results and to assist in administering the injection.

The number of sessions varies from **3** to **4** in accordance with the clinical response.
– All patients were instructed to undergo maintenance and functional recovery physiotherapy to be started at the end of the sessions.

TAB. 1

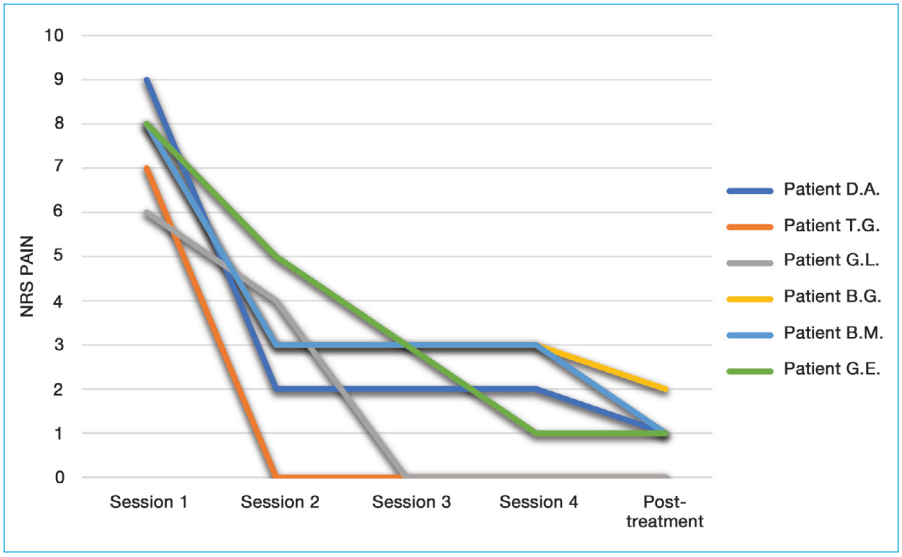
NRS (Numerical Rating Scale) of the 18 patients enrolled, before and after the treatment.

Patient	Age years	Gender	Pathology	Pain T0 (NRS scale)	Post-traumatic pain (NRS scale)
D.A.	76	M	Subacromion deltoid Bursitis	9	1
T.G.	76	F	Subacromion deltoid Bursitis	7	0
G.L.	69	F	Subacromion deltoid Bursitis	6	0
B.G.	59	M	Subacromion deltoid Bursitis	8	2
B.M.	67	F	Subacromion deltoid Bursitis	8	1
G.E.	30	F	Subacromion deltoid Bursitis	8	1
G.F.	40	M	Peritrochanteric Bursitis	6	0
B.S.	47	F	Peritrochanteric Bursitis	7	0
G.E.	40	F	Peritrochanteric Bursitis	7	2
M.N.	67	F	Peritrochanteric Bursitis	8	2
D.B.	48	F	Epicondylitis	9	1
M.D.	47	F	Epicondylitis	8	0
T.J.	54	F	Carpal tunnel Syndrome	9	1
B.M.	26	F	De Quervain's Syndrome	9	0
R.A.	58	M	Achilles Tendonitis	8	1
G.G.	49	M	Humeral biceps insertional Tendonitis	7	2
B.M.	67	F	Hypertrophic post-surgical scar	8	0
R.F.	74	M	Grade III Chondropathy and Gonarthrosis	9	1

Subacromion deltoid Bursitis

The treatment was carried out with **MD-Shoulder (intra-articular)**.

– The ultrasound-guided injection is performed after the injection of local anaesthetic (lidocaine or mepivacaine), 18G needle 1.25 x 40 mm. After entering the sub-acromion deltoidea bursa, 10 cc of physiological saline + 2 cc of anaesthetic are injected, a process that is useful to wash of the bursa, to detach the bursa germ layers and any fibrous lacinias. This is followed by injecting **MD-Shoulder**.
The patient is in a supine position with his/her limb lying down by their side and the intra-rotated hand clamped under his/her buttock; the needle is inserted into the side of the shoulder.



TAB. 2
Treatment outcome in Sub-acromion deltoid Bursitis.

Peritrochanteric Bursitis

As in the case of Sub-acromion deltoid Bursitis, an ultrasound-guided procedure is performed.
In this case with a 22G 0.7 x 90 mm needle, after local anaesthetic treatment with **MD-Tissue** (intra-articular).
The patient is lying on the contralateral side with respect to the site to be injected, with the lower limbs flexed.

Epicondylitis

In Epicondylitis, the tendon Tissue is frequently hypervascularised (FIG. 3).
Treatment was carried out with perinsertional tendon injections performed with 0.4-0.5 ml wheals in the epicondylar or epitrochlear site with **MD-Tissue**.

Carpal tunnel Syndrome

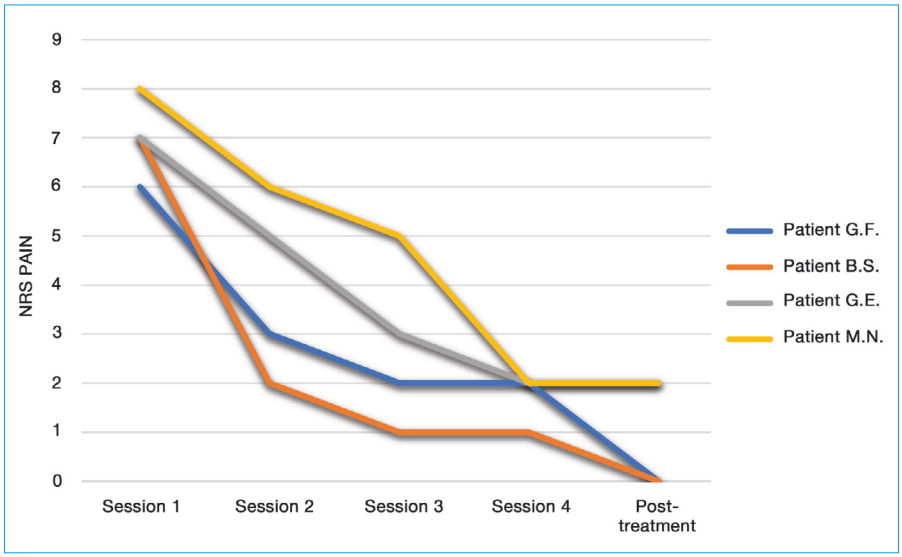
The treatment was carried out with **MD-Neural**.
With ultrasound assistance, the projection corresponding to the radial nerve is drawn on the skin with a special marker.
We then proceeded with injections with a 30G 0.30 x 13 mm needle along the course of the radial nerve at the wrist and carpus, injecting 0.4-0.5 ml into each wheal. The needle has an inclination of 45°/30° and is inserted 4-5 mm below the skin.

De Quervain's Syndrome

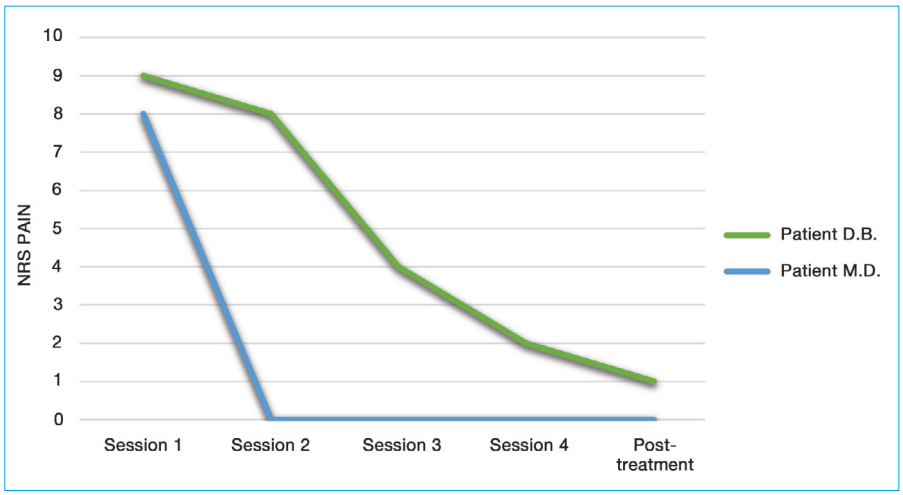
The treatment was carried out with **MD-Tissue**.
With ultrasound assistance, the projections corresponding to the tendon of the abductor longus muscle of the first finger are drawn on the skin with a special marker.
We then proceed with injections with a 30G 0.30 x 13 mm needle along the course of the tendon at the wrist, injecting 0.4-0.5 ml into each wheal. The needle inlet has an inclination of 45°/30°; the needle is inserted 4-5 mm below the skin.

Achilles Tendonitis

The injections were performed after the ultrasound examination in the lateral and

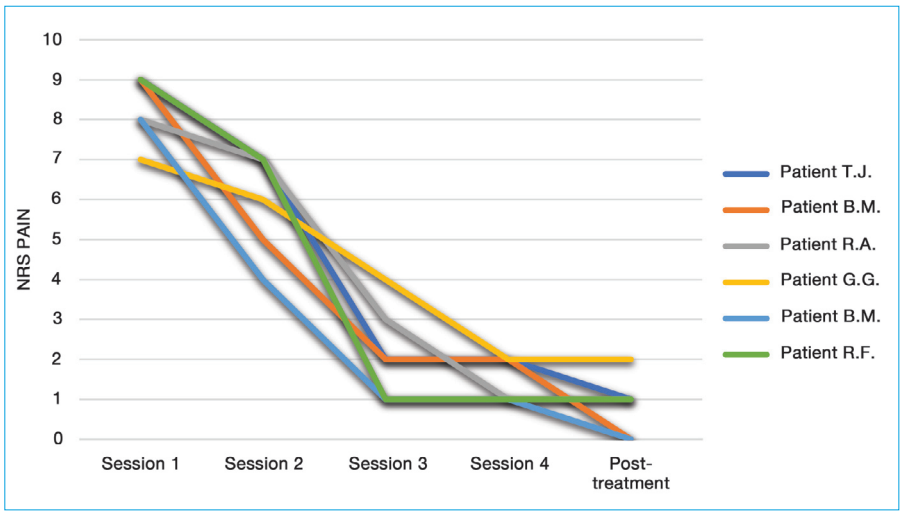


TAB. 3
Treatment outcome in Peritrochanteric Bursitis.



TAB. 4

Treatment outcome in Epicondylitis.



TAB. 5

Treatment outcome in Carpal Tunnel Syndrome, De Quervain's Syndrome, Achilles Tendonitis, Tendonitis of the biceps humeralis muscle on the radius, Post-surgical hypertrophic scar, Chondropathy and Gonarthrosis.

medial peritendinous site with **MD-Tissue** with a 30G 0.30 x 13 mm needle; the inclination was approximately 30°, inserting the needle to a depth of 4-5 mm.

Insertional Tendinitis humeral biceps muscle on the radius

In ultrasound-assisted mode, the radial insertion of the tendon of the humeral biceps muscle was drawn on the arm at elbow height, which showed an area of perinsertional effusion.
Some 0.4-0.5 ml wheals were made with **MD-Tissue**.

Post-surgical scar with painful pericatricial algic nodules

The patient presented with a painful, stiff left ankle scar with the presence of pericatricial nodules that appeared hypoechogenic on the ultrasound as if it were due to fibrous Tissue deposition.
In addition to pain, dysesthesia was present along the course of the scar up to the foot.
The treatment was carried out with **MD-Tissue**, a 30G 0.30 x 13 mm needle, with 0.4-0.5 ml wheals.

Chondropathy and Gonarthrosis

The patient, aged 74, had grade III Chondropathy and Gonarthrosis secondary to a medial meniscectomy performed 25 years earlier. A Backer's cyst was present and was emptied in ultrasound-guided mode during the first session.
The patient also had lymphoedema in his lower limbs.
The treatment with **MD-Knee** was extra-articular.
Injections were administered with a 30G 0.30 x 13 mm needle with 0.4-0.5 ml wheals in the lateral and medial site of the knee and in perirotuleal mode.

RESULTS

The mean NRS value of pain reported by the 18 patients at the time of diagnosis was **7.38** (max 9; min 6).

At the end of the treatments, all patients were contacted by telephone and reported clinical remission of their pain or a significant reduction in the most difficult cases or in long-lasting pain.
Reported pain was equal to **2.0** in 4 patients; equal to **1.0** in 7 patients; equal to **0** in 7 patients (TABB. 2-5).

Ultrasound scans repeated during the treatments showed a marked and progressive improvement in the clinical pictures, in particular as regards to the reduction of thickness of the subacromion deltoid bursa, the improvement of the elastonography and the reduction or disappearance of vascularisation in the inflamed tendons and structures.

No patients reported side effects or allergic reactions.

The treatment was well tolerated; in very few cases a localised burning sensation at the injection site lasting 2-3 minutes was reported.

– All patients expressed satisfaction with

the treatment, with the result obtained and because this result was achieved without the use of steroid drugs, which are known to have general and local adverse effects.

DISCUSSION

The clinical cases reported in this study show that treatment with the Collagen Medical Devices used guarantees a valid clinical and ultrasound response; it can therefore be included as an effective alternative to infiltrative therapies with corticosteroids, which are widely used for osteo-articular and musculo-tendon pathologies.
The side effects associated with the even local use of steroid drugs are still not widely studied, although they are objectively recognised. To date, there are no guidelines indicating the degree of systemic absorption, overall risk and/or local side effects (Alison *et* Al., 2019).

– The short-term use of steroids is associated with skin and electrolyte alterations, Hypertension, Hyperglycaemia, Pancreatitis, blood, immune and neuropsychological alterations; long-term use is correlated with much more serious consequences such as Osteoporosis, Aseptic joint necrosis, Adrenal insufficiency, Diabetes mellitus, Gastrointestinal, Hepatic and Ophthalmic Diseases, growth suppression and possible congenital malformations (Buchman, 2001).

The clinical complexity of some patients, their young age, or simply the risk of causing Tissue or systemic damage, open up the space for research into therapies such as those proposed here.

These have proven to be effective, free from adverse effects, easy to administer and repeatable without time constraints.

In particular, in the case of osteo-articular and musculoskeletal pathologies, we can rely on a safe, non-damaging and effective treatment protocol which can be suggested to patients who present pain and functional joint limitation and as maintenance after clinical remission.

– These treatments can be combined with a synergistic effect with physiotherapy or the use of drugs that the patient may already be taking, without contraindicating the good clinical response.

References

– Alison S. *et* Al. – Systemic Absorption and Side Effects of Locally Injected Glucocorticoids. Review PM, **2019** Apr; 11(4):409-419.
– Brandao R.A. *et* Al. – Efficacy and safety of a porcine collagen sponge for cranial neurosurgery: a prospective case-control study. World Neurosurf. **2013** Mar-Apr; 79(3-4):544-50.
– Buchman A.L. – Side effects of corticosteroid therapy. Review J Clin Gastroenterol. **2001** Oct; 33(4):289-94.
– Christensen B. *et* Al. – Effect of anti- inflammatory medication on the running-induced rise in patella tendon collagen synthesis in humans. J Appl Physiol (1985). **2011** Jan; 110(1): 137-41.
– Eming S.A. *et* Al. – Inflammation and metabolism in tissue repair and regeneration. Science. **2017** Jun 9; 356(6342):1026-1030.
– Friedrichs J., *et* Al. – Cellular remodelling of individual collagen fibrils visualized by time-lapse AFM. J Mol Biol. **2007** Sep 21; 372(3): 594-607.
– Fung D.T. *et* Al. – Second harmonic generation imaging and Fourier transform spectral analysis reveal damage in fatigue-loaded tendons. Ann Biomed Eng. **2010** May; 38(5): 1741-51.
– Hussain A.I. *et* Al. – Citrullus colocyntihis (L.) Schrad (bitter apple fruit): a review of its phytochemistry, pharmacology, traditional uses and nutritional potential. J Ethnopharmacol. **2014** Aug 8; 155(1):54-66.
– Keil S.D. *et* Al. – Inactivation of Plasmodium spp. in plasma and platelet concentrates using riboflavin and ultraviolet light. Transfusion. **2013** Oct; 53(10): 2278-86.
– Masaki H. – Role of antioxidants in the skin: anti-aging effects. J Dermatol Sci. **2010** May; 58(2):85-90.
– Milani L. – A new and refined injectable treatment for muscoloskeletal disorder. Bioscaffold properties of collagen and its clinical use. Physiological Regulating Medicine **2010**/1; 3-15.
– Nawash O. *et* Al. – Ethnobotanical study of medicinal plants commonly used by local Bedouins in the Badia region of Jordan. J Ethnopharmacol. **2013** Jul 30; 148(3):921-5.
– Ngamphaiboon N. *et* Al. – Pyridoxine treatment of vincrestine-induced cranial polyneuropathy in an adult patient with acute lymphocynetic leukemia: case report and review of the literature. Leuk Res. **2010** Aug; 34(8):194-6.
– Ottaviani M. – Trattamento delle patologie articolari con Collagen Medical Devices. - Studio clinico su 257 pazienti. La Med. Biol. **2014**/3; 11-21.
– Rahman A.U. *et* Al. – Anti-inflammatory isoflavonoids from the rhizomes of *Iris germanica*. J Ethnopharmacol. **2003** Jun; 86(3-2):177-80.
– Randelli F. *et* Al. – Caratterizzazione del *turnover* del collagene in tenociti umani coltivati su matrice di collagene MD-Tissue. La Med. Biol., **2018**/4; 67-68.
– Schutz C. *et* Al. – Profiling of isoflavonoids in *Iris germanica* rhizome extracts by microprobe NMR and HPLC-PDA-MS analysis. Fitoterapia. **2011** Oct; 82(7):1021-6.
– Silbernagel K.G. *et* Al. – The majority of patients with Achilles tendinopathy recover fully when treated with exercise alone: a 5-year follow-up. Am J Sports Med. **2011** Mar; 39(3):607-13.
– Wenger M.P.E. *et* Al. – Mechanical Properties of Collagen Fibrils. Biophys J., **2007** Aug 15; 93(4):1255-1263.
– Wilson J.X. – Mechanism of action of vitamin C in sepsis: ascorbate modulates redox signaling in endothelium. Biofactors. **2009** Jan-Feb; 35(1):5-13.
– Zhao C. *et* Al. – Structure of Collagen. Methods Mol Biol. **2021**; 2347:17-25.

Author

Dr. med. Elena Guidi

– Expert Doctor in Advanced and Interventional Musculoskeletal Ultrasounds
– General Sonographer (SIEMG degree)

Via F. Filzi, 4A
I – 41016 Rovereto sulla Secchia - Novi di Modena (MO)