revista de ciências da saúde NOVA ESPERANÇA VOLUME 21- NÚMERO ESPECIAL MOSSORÓ- 2023
ISSN ELETRÔNICO 2317-7160

USE OF COLD ATMOSPHERIC PLASMA TO TREAT SKIN WOUNDS: A SYSTEMATIC REVIEW

USO DE PLASMA ATMOSFÉRICO FRIO NO TRATAMENTO DE FERIDAS DE PELE: UMA REVISÃO SISTEMÁTICA

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Abstract. Cold Atmospheric Plasma (CAP) has emerged as a promising technology for the wound healing process. Therefore, this study aimed to make a systematic review of CAP effects on the healing of skin wounds, focusing on the protocols used and decision-making for applications. For its development, two researchers conducted a blind search in five electronic databases (PubMed; Web of Science; Scielo; Scopus, and EMBASE) using a series of keyword combinations. The first selection was by the title, the second by the abstract, and the third by the full reading of the article. To compose this study, 28 articles were selected. The greatest difficulty was the non-uniformity of the keywords related to CAP and intervention protocols not described systematically and clearly. There is an agreement among the authors that CAP contributes positively through reactive species, nitric oxide, proinflammatory, and cellular signaling, both quantitatively and qualitatively to the healing process, acting in the re-epithelialization, angiogenesis, increased oxygenation, coagulation, collagen synthesis, among others. The phase it operates and the ideal application time are still controversial. Its application can occur directly and indirectly, with most studies still being conducted in the laboratory in acute and clean wounds. The most commonly used gases are Helium and Argon. It is concluded that the positive effect of CAP on the healing process is already a consensus in the literature. However, there is still a need to clarify the mechanisms involved.

Keywords: Plasma gases; Skin; Wound Healing

Resumo. O Plasma Atmosférico Frio (CAP) surgiu como uma tecnologia promissora para o processo de cicatrização de feridas. Diante disto, este estudo teve como objetivo fazer uma revisão sistemática dos efeitos do CAP na cicatrização de feridas cutâneas, com foco nos protocolos utilizados e na tomada de decisão para aplicações. Para seu desenvolvimento, dois pesquisadores realizaram uma busca cega em cinco bases de dados eletrônicas (PubMed; Web of Science; Scielo; Scopus e EMBASE) usando uma série de combinações de palavras-chave. A primeira seleção foi pelo título, a segunda pelo resumo e a terceira pela leitura completa do artigo. Para compor este estudo, foram selecionados 28 artigos. A maior dificuldade foi a não uniformidade das palavras-chave relacionadas ao CAP e os protocolos de intervenção não descritos de forma sistemática e clara. Há concordância entre os autores de que o CAP contribui positivamente por meio de espécies reativas, óxido nítrico, pró-inflamatório e sinalização celular, tanto quantitativa quanto qualitativamente para o processo cicatricial, atuando na reepitelização, angiogênese, aumento da oxigenação, coagulação, síntese de colágeno, entre outros. A fase em que atua e o tempo ideal de aplicação ainda são controversos. Sua aplicação pode ocorrer de forma direta e indireta, sendo a maioria dos estudos ainda realizados em laboratório em feridas agudas e limpas. Os gases mais usados são o hélio e o argônio. Conclui-se que o efeito positivo do CAP no processo cicatricial já é consenso na literatura. No entanto, ainda há a necessidade de esclarecer os mecanismos envolvidos.

Palavras-Chave: Gases plasmáticos; Pele; Cicatrização de feridas.

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Submissão: 25/10/2023 Aceite: 25/10/2023
DOI:10.17695/rcsne.vol24.nEsp..p667-685

INTRODUÇÃO

Even with the natural cycle, wound treatment is a challenge, especially in ensuring the uniformity and effectiveness of the repair process. Many mechanisms have been used in this context, such as topical drugs and even therapies with non-invasive technologies. Among these technologies, there is the use of cold atmospheric plasma (CAP) generated by discharges between two electrodes separated by an insulating dielectric barrier (DBD) that energizes air[1]. In the treatment of skin wound healing, the use of this device has been evaluated among the non-invasive procedures with benefits in the acute phase since it has an antiseptic effect to restore the physiological potential of healing by decreasing bacterial load; and stimulating the tissue regeneration and healing process, with effects on the proliferation of endothelial cells that point to the possibility of positive impacts on angiogenesis mediated by these cells. Besides, there is the easy application and good acceptance by patients[2,4], low cost, the possibility of using various gases makes it more versatile, the most used being argon and helium, which still raises discussion as to the choice[5].

In this perspective, the use of CAP arouses the interest of the medical sciences, as it has been considered tolerable by biological tissues, and its ability to inactivate microorganisms and promote a change in tissue without causing inflammation. These properties make it an interesting alternative for wound healing treatment and are promising for use in clinical applications. Nevertheless, despite the knowledge about its benefits and effects on the wound healing process, there is still a need to understand the mechanisms of how CAP works, as well as well-founded justifications for choosing the gas, definition, and consolidation of the parameters to be used and protocols to be followed[6].

Therefore, this study aimed to conduct a systematic review of the effects of cold atmospheric plasma on the healing of skin wounds, focusing on the protocols used and decision-making for applications.

METHODS

Research Strategy

Blindly, two researchers conducted an electronic search in the following databases: PubMed; Web of Science; Scielo; Scopus, and EMBASE using the following keyword combinations: “Cold Atmospheric Plasma”; “Skin”; “wound healing” and “Non-thermal

Atmospheric Pressure plasma"; "Skin"; "wound healing" and "CAP"; "Wounds"; "Healing" and, whenever necessary, some combination of these words were applied in an attempt to find as many articles as possible. The same keywords were used in English and Portuguese depending on the researched database. Preference was given to the use of those registered in the health descriptors (DECS) and in the Medical Subject Headings (MESH). However, other words, based on the articles found, were used whenever it was impossible to use only registered descriptors.

The guiding question for this study was: What is the effect of cold atmospheric plasma on skin wound healing?

Study selection

For the inclusion criteria, the language was considered, and searches were conducted in Portuguese and English with the respective descriptors. The temporal space established was from the previous 12 years, a period when studies with plasma medicine emerged; the article being available in full, being published in an indexed journal, being an original article with experimental studies in vivo; dealing with the process of tissue repair in skin wounds was also considered. The exclusion criteria were applied to those articles that dealt with the use of cold atmospheric plasma for other purposes, such as: exclusively to decrease the bacterial load or treatment of wounds associated with diabetes, oncology area, dental treatments, surface treatment, grafts or flaps, those who worked with humans with comorbidities, among others; in addition to those studies that used another type of plasma to treat skin wounds or performed overlapping plasma with adjuvant treatment. We also excluded letters to reviewers, review studies, as well as academic final works, dissertations, and theses. Studies that worked exclusively with in vitro and ex vivo assays were also excluded.

The researchers were careful to ensure that all articles met the inclusion criteria. In cases of discrepancies between articles, all criteria were reviewed and discussed among researchers. After this first search, the articles were selected according to the criteria mentioned above. The first selection was made by the title, the second by the abstract, and the third by the full reading of the article. Thus, after applying the inclusion and exclusion criteria, the article was selected, and the necessary information was extracted, which is presented in the results.

RESULTS

The search results according to the keywords used, their combinations, and databases are summarized in Table 1. Scopus and PubMed generated the most results. Most of the articles selected for the present study were initially found in the latter, and from it, the repetitions were excluded. When the research was conducted in Portuguese, no study was found. Therefore, all articles selected for the present study were in English. There was the exclusion of articles by repetition between the databases and keyword combinations. The articles were also excluded, based on the inclusion and exclusion criteria by title, abstract, and full-text reading. Finally, of the 627 articles found, 29 were selected to compose the study base of this review.

TABLE 1: DETAILED SEARCH RESULTS IN EACH DATABASE ACCORDING TO THE KEYWORDS USED.

Database	Search	1	2	3	4	5	6	Total[b]
Scopus	Total number	101	35	81				217
	Selected number	5	5	7				17
	Repeated number	0	0	2				2
EMBASE	Total number				45			45
	Selected number				5			5
	Repeated number				8			8
Web Of Science	Total number		74	78				152
	Selected number		4	13				17
	Repeated number		4	14				18
PubMed	Total number		83			81	49	213
	Selected number		25			4	0	29
	Repeated number		0			23	10	33
	Number of papers selected[c]							29

[a]1- CAP, Skin, Wound; 2- Non-Thermal Atmospheric Pressure Plasma, Skin, Wound; 3- Cold Atmospheric Plasma, Skin, Wound Healing; 4- CAP, Wound, Healing; 5- Cold Atmospheric Plasma, Skin, Wound; 6- CAP, Skin, Wound healing. bTotal number of found articles may be greater than the number of papers used since the same paper appears multiple times in the same database with different keywords. cFinal number of papers selected.

Table 2 shows the general information of the articles from the delimitation of the last 12 years of publication. There has been a publication trend over the years, and in 2014 there was no publication on the use of CAP for wound healing as an original article. And in 2023 there have been no publications within the criteria established by this research yet. According to the criteria, only one literature review was found on the topic, which was excluded. Germany stands out as the country with the most publications, followed by China, with a predominance of study groups that are constantly being found and cited in the articles.

One factor that caught our attention was the diversity of keywords related to cold atmospheric plasma used among the articles, even within the same study group or within the same journal. This lack of standardization, in a way, hinders research within the area and the technology consolidation in the scientific world, as it ends up not establishing an identity in the scientific community. Therefore, it is always necessary to perform various combinations to locate articles on the subject, making it difficult to locate them.

Of these keywords, the most used in the studies, as shown in Table II, was "Cold Atmospheric Plasma (CAP)". Even though there is a trend in the area for its use, the only ones that presented some relationship when the consultation was performed in Decs and Mesh were: "Cold plasma"; "Nonthermal plasma"; "Non-thermal atmospheric pressure plasma". All of them presented the related term "Plasma gases".

Still, regarding the publication characteristics, there is an interest in the scientific community concerning publications in the area. Almost all the articles found are in journals with high-impact factors.

TABLE 2: GENERAL INFORMATION ON ARTICLES AND JOURNAL

References	# of authors	País	Journal	IF	Year	Keyword
Arndt S. et al.	11	Germany	PLOS ONE	2.74	2013	Cold atmospheric plasma (CAP)
Isbary G. et al.	14	Germany	Clinical Plasma Medicine	5.80	2013	Plasma Cold atmospheric plasma (CAP)
Garcia-Alcantara et al.	12	Mexico	Archives of Medical Research	N/I	2013	Cold plasma, Plasma treatment
Wu et al.	21	Philadelphia	Journal of Surgical Research	2.771	2013	FE-DBD plasma; Non thermal plasma
Arndt et al.	10	Germany	PLOS ONE	2.74	2015	Cold atmospheric plasma (CAP)

Xu et al.	8	China	Wound Repair Regeneration	2.84	2015	Cold plasma
Lee et al.	9	Republic of Korea	Journal of tissue engineering and regenerative medicine	3.180	2016	non-thermal; microplasma
Akimoto et al.	9	Japan	Archives of Biochemistry and Biophysics	3.391	2016	Low-temperature plasma
Kang et al.	10	Korea	Experimental Dermatology	3.368	2016	Non-thermal atmospheric pressure plasma
Schmidt et al.	5	Germany	Experimental Dermatology	3.368	2017	Plasma medicine
Rad; Davani	2	Iran	Review of scientific instruments	1.74	2017	Dielectric Barrier Discharge (DBD); Plasma
Choi et al.	6	Korea	Scientific reports	3.998	2017	Non-thermal plasma (NTP)
Kubinova et al.	7	Prague	Scientific reports	3.998	2017	Non-thermal plasma (NTP)
Lin et al.	9	China	Plasma Medicine	5.74	2017	Atmospheric-pressure plasma jet (APPJ)
Arndt s. et al.	5	Germany	Journal of Dermatological Science	4.01	2018	Cold atmospheric plasma
Chatrai et al.	5	Iran	Scientific reports	3.998	2018	Non-thermal atmospheric plasma
Duchesne et al.	5	France	Plasma Medicine	5.74	2018	Cold atmospheric plasma; plasma-activated media
Rad; Davani; Etaati	3	Iran	AustralasianPhysical & Engineering Sciences in Medicine	1.10	2018	Helium plasma jet · Atmospheric pressure plasma
Shi et al.	9	China	Current medical science	1.273	2018	Low Temparature plasma (LTP)

Zhang et al.	9	China	Contributions to Plasma Physics	1.226	2019	Cold atmospheric plasma
Schmidt et al.	5	Germany	Theranostics	8.579	2019	Plasma medicine
Shekhter et al.	9	Russia	European Journal of Pharmaceutical Sciences	3.616	2019	Plasma-chemical NO generation NO-containing gas flow
Cui et al.	6	Korea	International Journal of Molecular Sciences	4.210	2020	Low-temperature plasma (LTP)
Xu D. et al.	9	China	Microorganisms	N/C	2020	plasma-activated water
Hamed; Mashhadani; Jassim	3	Iraq	International Journal of Research in Pharmaceutical Sciences	0.60	2020	Non-thermal atmospheric pressure plasmajet(NAPPJ), argon plasma jet,
Lou et al.	8	Taiwan	Frontiers in Bioengineering and Biotechnology in Bioengineering and Biotechnology	5.48	2020	cold atmospheric plasma jet
Moelleken et al.	6	Germany	journal of the german society of dermatology	0.79	2020	Cold atmospheric plasma (CAP)
Schmid et al.	4	Germany	IEEE transactions on radiation and plasma medical sciences	1.309	2021	Plasma medicine
Landscheidt et al.	5		Advaces in skin& Wound Care	1.13	2022	Active wound dressing, chronic wound, cold atmospheric plasma, plasma medicine, wound healing, wound infection

+N/I – Not Included. IF - Impact factor

From the data above, Table 3 describes the methodologies used in the articles, from the guinea pig used, which focused on mice and rats; to the technique for wound formation and the type of wound, in addition to some descriptions of the device and application characteristics, following the sequence of articles of table 2. In general, the articles point to a predominance of experimental studies in laboratories with few reports of clinical applications for treating wounds, such as those applied in humans.

Due to the device's versatility for applying cold atmospheric plasma, there is still a tendency to build it in the study group, with the use of commercial devices only in articles from Germany, with technology devices manufactured in this country. In a way, this tends to variability in the form of description and choice of parameters to be used.

TABLE 3: DESCRIPTION OF PROTOCOLS AND METHODOLOGIES USED IN THE ARTICLE.

Article	Model	Wound Formation	Type of wound	Application	Equipment	Mode	Used gas	Application phase
01	Wild mice	Punch 6mm	Acute and clean	Direct	MicroPlaSter ßH	Pulsed	Argon	Not reported
02	Humans	Spontaneous	Chronic and infected	Direct	MicroPlaSter alpha device	Pulsed	Argon	Not reported
03	Mice	Not reported	Acute and clean	Direct	Created by the group	Pulsed	Argon e Helium	Immediately after the wound
04	Yorkshire pigs	Electric dermatome	Acute and clean	Direct	Not reported	Pulsed	Not reported	Immediately after the wound
05	Mice	Not reported	Acute and clean	Not reported	Micro PlaSter ß	Pulsed	Argon	Not reported
06	Mice BALB	Punch hole	Acute and clean a	Direct	Created by the group	Pulsed	Argon	Immediately after the wound
07	Sprague-Dawley Rats	Burn	Acute and clean	Direct	Created by the group Microplasma jet	Pulsed	Helium	Not reported
08	Mice	Scalpel and Scissors	Acute and clean	Direct	Created by the group	Pulsed	Helium	Not reported
09	Sprague-Dawley Rats	Punch 8mm	Acute and clean	Direct	Created by the group	Continuous	Helium	Not reported
10	Mice	Punch 6mm	Acute and clean	Direct	Created by the group	Pulsed	Not reported	24 hours after the wound
11	Mice	Punch 6mm	Acute and clean	Direct	MicroPlaSter ß	Pulsed	Argon	Not reported
12	Mouse	Punch 6mm	Acute and clean	Direct	Created by the group	Pulsed	Argon	Immediately after the wound
13	Mouse Wistar	Punch 8mm	Acute and clean	Direct	Created by the group	Continuous	Ambient air	Immediately after the wound
14	Mouse	Punch 8mm and scissor	Acute and clean	Direct	Created by the group	Pulsed	G3: Argon G4: Argon + Ambient air	Immediately after the wound
15	Immunocompetent mice	Micro scissors	Acute and clean	Direct	kINPen 11	Pulsed	Argon	Immediately after the wound
16	Mouse	Pressure with magnets	Chronic and clean	Direct	Created by the group	Pulsed	Helium	48 hours after the wound
17	Mice	Biopsy Punch 6mm	Acute and clean	Direct and indirect	Created by the group	Pulsed	Helium	Not reported

18	Mouse	Dermatological punch	Acute and clean	Direct	Created by the group	Pulsed	Helium	Not reported
19	Mice	Punch	Acute and clean	Direct	Created by the group	Pulsed	Argon	Immediately after the wound
20	Sprague-Dawley Rats	Blade 1,3 cm x 1,3 cm	Acute and clean	Direct	Created by the group	Pulsed	Helium	Not reported
21	Immunocompetent mice	Micro scissors	Acute and clean	Direct	kINPen 11	Pulsed	Argon	Immediately after the wound
22	Wistar rats	Surgical incision 8 a 10 mm	Acute and clean	Direct	Created by the group	Continuous	G1: Ambient air G2: NO	24 hours after the wound
23	Mice	Punch 12mm	Acute and clean	Direct	Not reported	Pulsed	Helium + ambient air	Not reported
24	Mice	Surgical scissors 2 cm	Acute and infect	Indirect	Created by the group	Pulsed	Not reported	Immediately after the wound
25	Mice	Not reported	Acute and clean	Direct	Not reported	Continuous	Argon	Immediately after the wound
26	Mouse	Not reported	Acute and clean	Direct	Not reported	Pulsed	G1: Helium G2: Helium + Argon	Not reported
27	Human	Spontaneous	Chronic and infected	Direct	SteriPlas®	Continuous	Argon	Only in chronic wounds
28	Mice	Surgical wound	Acute and clean	Direct	kINPen MED	Continuous	Argon	Immediately after the wound
29	Humans	Chronic Wound	Chronic and infected	Indirect	Not Reported	Not reported	Not reported	Not reported

The application in most articles was direct and in acute and clean wounds. Thus, the study of chronic wounds is still incipient and deserves attention from researchers in the area. None of the articles described the pulsed or continuous mode in this way. However, for classification purposes, in this article, the pulsed mode was considered for those who reported the frequency within the parameters, and for those who did not bring this data, the continuous mode.

The application phase was not reported in many articles, and for those which reported, the application immediately after the wound predominated and was direct. Only three articles reported indirect application, one of which used plasma-activated water to treat wounds that used a plasma-activated medium.

Concerning the time of application, the information in the articles was very scarce. None of the selected articles brought the angle between the pen and the skin used at the time of application. On the distance from the skin, some did not report, others brought an average distance, and only article 09 reported using a spacer to ensure exact distance from the skin.

Because the information among the articles is very diffuse and the gaps left by the detailing of information, the attempt to perform statistical analysis with the data was unsuccessful. Therefore, it was decided to maintain only the systematic review. This points to a problem in the area because it hinders the understanding and reproducibility of research to strengthen the application of CAP.

DISCUSSION

The effect of cold atmospheric plasma (CAP) treatment in promoting faster and better-quality healing, either alone or in association with other therapies such as stem cells, has been widely discussed. However, the mechanism of action, parameters, and modes of application of CAP are not yet elucidated and consolidated[1,5,9].

Plasma medicine emerged from the possibility of cooling low-pressure plasma thus making it safe for use in biological studies. There are reports that skin temperature during treatment ranged between 33 and 41°C [5, 10, 11, 12, 13], with variations at the time of application between 41°C in the treatment with argon and 33°C with helium gas in the first 8 to 10 seconds[5], and an average of 37°C after 4 minutes of application and slightly above 40°C after 10 minutes10. Thus, the literature indicates that CAP does not cause any skin damage or any other side effect, with good tolerance for application in human volunteer patients[11-13].

However, in a study on pig skins, the authors drew attention to caution regarding power, reporting that there was burn in the case of higher power (0.31 W / cm²), in contrast to the lower power (0.15 W / cm²). Thus, the authors concluded that exposure to high-power CAP for more than one minute is likely to cause damage to the wounded skin. Nevertheless, only the application was made directly, without specifying the form of application or the device used[14].

Of all the studies analyzed, only one stated that neither direct nor indirect treatment with CAP effectively accelerated wound closure when histologically analyzed on day 7. These authors used a time of 10 and 30 seconds in a single or repeated treatment three times with helium gas, with a device created by the group8. However, there is already a consensus in the literature that CAP promotes an improvement. This may be related to reactive oxygen and nitrogen species that, in correct doses, are fundamental in this process.

This can be proven in studies that evaluated the expression of a key regulatory antioxidant defense protein, Nrf2; and stated that the toxicity resulting from exposure to reactive plasma-derived species is negligible. Furthermore, the authors also demonstrated qualitatively and quantitatively that there were no increased levels or increased phosphorylation of histone A2X, a marker of DNA damage and repair[15-18].

Some report that this effect on healing is secondary to the antibacterial effect of CAP, preventing contamination, which would delay the process. However, studies have shown a higher rate of retraction and re-epithelialization of clean wounds treated with CAP[19, 20,21]. Schmidt[18] also quantitatively evaluated this effect on healing through five parameters: collagen pattern (distribution, fiber orientation, early and mature collagen, amount of collagen), inflammation (inflammatory infiltrate), granulation (tissue granulation, cell accumulation, cell migration), angiogenesis (blood vessel formation, vascularization), and apoptosis (blistering, shrinkage, condensation).

Another association found was with the effect and duration of healing as dose-dependent. The longer the treatment time, the faster the healing is, and the increase in gas velocity also increases the amount of reactive species and accelerated healing[22]. However, Lou[20], when comparing the effect of the application for 1, 3, and 5 minutes, concluded that 1 minute was the most efficient in wound closure. In agreement with this, reports in the literature state that treatment for short periods (10 – 40 seconds) promotes wound healing, while long treatment times (50 seconds) suppress healing[23]. When comparing 10 and 30 seconds of treatment, the 30s group showed improvement in healing through the migration of endothelial cells and collagen production24. In agreement with this, another study that compared wounds treated for 30, 40, and 50 s also observed a significant difference between the 30 s group and the control group, which contributed to the conclusion that the time of 30s was considered the appropriate treatment time[12]. Time is still one of the most controversial parameters in plasma treatment, and more systematic studies are needed to define ideal parameters[23].

Regarding the application interval, the effect of CAP in the treatment of chronic wounds was observed in 37 human patients. Therapy resulted in a reduction in wound area of 63.0% for Group 1 (treated once a week) and 46.8% for Group 2 (treated 3 times a week). In contrast, the wound area of the placebo group increased by 17.5%. At baseline, the mean wound depth was 2.4 mm in Group 1; 2.8 mm in Group 2, and 2.6 mm in the placebo group. Throughout the treatment, wound depth was reduced by 53.5% in Group 1, 16.6% in Group 2, and 34.2% in the placebo group. Another important finding reported by the volunteers in this study was that pain relief after seven and twelve weeks was statistically significant, which was not observed in the placebo group[25].

In addition to time, there is a controversial discussion regarding the moment that CAP effectively contributes to the healing process. There were reports of these effects being well pronounced from the early stages of healing to the end[24], with the presence of edema in the wounds of the control groups, in contrast to those treated with only a small amount of exudate and fibrin[26]; and significantly accelerated closing of the treated wound since day 3, with almost complete re-epithelialization (92-98%) on day 12 and without scar formation18. In agreement with this, in another study, there was the formation of crusts already on day 3 for the treated groups while the control wounds were still inflamed[12]. Another study found significant improvements on days 3 and 5. After day 15, both the treated and control group wounds closed equally. Still, there was an increase in the number of macrophages in those treated, fundamental to promoting cleaning of the wound while secreting important growth factors for healing. It was also possible to observe that on day 5, there was an induction of 30% of neutrophils compared to the placebo group, which was not observed on day 15 and leads to the belief that CAP induces this migration at the acute moment of the healing process[15].

Still, from this perspective, there are reports that CAP has the potential for wound healing in the remodeling phase11; and that macroscopically the treated wounds close almost completely on day 10, in contrast to the controls that close only on day 14, with no effect in the early stages[1].

In this regard, another study[27] observed significant wound reduction in treated animals from day 7 onwards. It is worth mentioning that this reduction in the wound area is related to histological improvements that are directly related to better re-epithelialization and vascularization, which involves neovascularization and regeneration of vessels; tissue oxygenation, stimulation of proinflammatory signaling, and greater production and orientation and type of collagen (I and III), with no disagreement between the authors on these aspects[7,12,15,18,26,28,33].

Regarding the microscopic characterization of wounds treated by CAP, there is an almost complete recovery of the epidermis, with stratum corneum, hair follicles, and sebaceous glands in those treated and a higher density of collagen and regenerated muscle tissue. In contrast, the control wounds remained with a thin epidermis, low collagen density, and damaged muscle layer. Thus, the treatment-induced repeatability increases the proliferation of keratinocytes and accelerates collagen fibers' regeneration through effects on fibroblasts[27,30].

Another important factor is that CAP does not activate apoptotic mechanisms in keratinocytes[28,29], while potentially activating the NF-kB signaling pathway in fibroblasts, modulating cell cycle progression, promoting cell proliferation, and eventually facilitating wound healing[31].

The time of treatment of wounds seems to influence the production of collagen because when comparing wounds treated for 30 and 50 seconds, and evaluated by immunofluorescence for vimentin (fibroblast marker, which indicates the proliferation of these) and type I collagen (indicates the ability of fibroblasts to synthesize collagen) there was a significantly higher expression in wounds for 30s. Type I collagen was significantly higher in the 30s group and significantly lower in the 50s group when compared to the control group[31]. However, disagreeing with this, a study[11] found no significant difference between the groups regarding the arrangement of collagen bundles. Some authors reported that in both groups, control and treated, collagen was disordered, and no difference could be found in the diameter of the bundles in the wound tissues, which indicated that CAP did not affect collagen deposition[21].

An interesting factor that arouses a relevant discussion about the use of several devices in the practice of cold atmospheric plasma was raised in the studies. Some authors compared the effect of different devices with collagen fibers and found differences in the type and deposition depending on the device used[26].

Some authors have reported specific factors such as proteins influencing healing; such as the presence and action of galectins, important proteins in cell adhesion, differentiation, transcription, and consequently in healing, with an accelerating role in this process; and compared the use of CAP with an electrocoagulator, being possible to find an increase of galectins-1, -2, and, -3 in wounds treated with plasma in contrast to a decrease in electrocoagulation, which indicates that treatment with CAP is more recommended in wound healing[32].

It is noteworthy that most studies demonstrate results from experiments performed with acute and clean wounds. However, Isbary[13] reported that CAP, when applied to chronic wounds of different etiologies and with the treatment of 3 to 7 minutes, had a beneficial effect on healing these wounds, especially for chronic venous ulcers. With improvement in some aspects, either length or width, which generally did not affect the area as a whole.

Still, there is a relevant discussion regarding the type of wound, since most studies, laboratory-based, follow the tendency of small wounds, made with a punch. Nevertheless, when comparing the healing of smaller and larger wounds, they have different behavior. The tendency of wound healing is especially pronounced in the latter, which showed better results when compared with smaller ones. However, the authors clarify the need for more investment in studies on this perspective[10].

Regarding the choice of gas, there is a comparison in the literature of the action of argon and helium in the wound with a better result for the latter that demonstrated more efficiency in the healing process with the promotion of angiogenesis and repair of existing blood vessels, in addition to the activation of platelet factors for the coagulation process in the wound. This is attributed to nitric oxide production in its jet which was not found in argon5. However, the mixture of argon with 0.4% oxygen addition contributed to the faster wound healing10. Likewise, some results point to a more significant improvement in healing with the mixture of helium and Argon gases compared to treatment with helium gas alone. In this case, a decrease in the infiltration of inflammatory cells around the wounds was found, and the presence of markedly increased granulation16 and epithelialization tissue after day 7[20].

Regarding the forms of application, direct or indirect, some reports did not find significant results for both methods[8]. Disagreeing with this, a study by indirect treatment of plasma-activated water reported significant improvement of the treated wound, in addition to which, on day 17, they were fully healed, unlike the control. Thus, the authors concluded that the healing time for those wounds treated indirectly was significantly shorter when compared to the control group. In histology, the number of inflammatory cells was also significantly lower in the treated group, causing less severe inflammatory damage to the tissue and consequently accelerating healing[7].

FINAL CONSIDERATIONS

Cold atmospheric plasma promotes healing with qualitative and quantitative superiority concerning the number of cells, proinflammatory signals, collagen fibers, re-epithelialization, and vascularization, which impacts the reduction of healing time compared to its control group. There are reports that time and speed directly influence these results, with reactive oxygen species and nitric oxide being key points in this process and some proteins and fibroblast signaling, keratinocytes involved.

Nonetheless, despite this consensus, the authors always point to a need for a better understanding of the mechanisms involved in these results, in addition to which there is still a lack of standardization regarding the parameters and forms of use, and the literature points to a need for better systematization and exposure of the methodology so that it is reproducible and thus can contribute to the advancement of research within plasma medicine.

In addition, there is still a need for more clinical applications, extrapolating the laboratory environment, with different types of wounds and forms of application, concerning the direct and indirect mode, so that there is better clarification on how CAP acts in different contexts. Therefore, this systematic review raises the need for standardization and diversity of applications to contribute to the consolidation of this technology in the wound healing process.

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