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Chitosan-based films for wound healing applications: A meta-analysis to access the impact on wound closure rate

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Abstract: Wound healing novel materials have been emerging as a hot topic, as wounds are reaching epidemic proportions, with both economic and psychosocial consequences. Chitosan-based films increase the rate of re-epithelialization and wound closure rate which, due to their antimicrobial properties, should contribute to wound closure. With the constant raise of scientific publications regarding this subject, we aimed to establish a direct relation of chitosan films on wound closure rate based on recent data. The available literature was gathered from Scopus database, followed by a bibliometric analysis. Results were sorted based on wound closure metrics and statistical information. The data extracted from the selected articles were analyzed and compared regarding the wound closure rate measured in animal models. The results showed good homogeneity of positive results among the studies selected. On the other hand, due to unstandardized parameters in chitosan films formulations among the articles studied here, no correlation with chitosan concentration in wound dressing films with the wound closure effect could be observed. However, further investigations of such correlation with clinical tests are still needed. In this context, this work demonstrated consistency in the positive impact of chitosan films on wound healing, demonstrating that chitosan films have the potential for biomedical use and their research is justified. The results of this work might help to understand the relationship between the actual contribution of chitosan and in vivo wound healing evaluation, and provide a reference to new approaches for the treatment chronic wounds.

Keywords: Chitosan films, wound healing, wound closure rate, Data mining, Statistical analysis, Bibliometrics.

Adherence to the BJEDIS' scope: This work is closely related to the scope of BJEDIS as it presents bibliometric research and meta-analysis regarding the application of Chitosan films on wound healing products

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1. INTRODUCTION

Several pathological conditions can result in the development of chronic wound, including arterial or venous insufficiency, diabetes, excessive skin pressure, presence of exogenous objects and infection (1). The incidence of such chronic wounds (venous, diabetic foot or pressure ulcers) is increasing and reaching epidemic proportions, which results in substantial economic and psychosocial costs (2,3).

Skin wound repair is a complex biological process involving events of orchestrated cell signaling and well- defined biochemical cascades. These events occur sequentially, overlapping phases ranging from hemostasis, inflammation, proliferation and remodeling in response to the lesion and its microenvironment (1,4). The critical points for wound treatment are: (I) maintaining environmental humidity, (II) preventing or treating infection, and (III) minimizing skin irritation or friction between the wound and clothings or wheelchair devices, pillows and mattresses (5). Currently, there are many products commercially available and used for wound healing, although little evidence supports their use. However, the major limitation with traditional dressings (gauze and cotton compounds) and an ordinary formulation such as cream or gels are the short residence time, leaks, poor patient compliance, less preservation of the moist environment (1,3).

In general, many dressings have been developed to try to protect the healing wound from infection (6,7) and also to help promote the healing process itself (8). Chitosan-based films, besides being naturally bioadhesive preparations, provide moist occlusion, which support the inflammatory phase by creating an environment with low oxygen tension, increasing the rate of re-epithelialization and wound closure rate. Moreover, regarding the infection prevention and treatment, chitosan is often recognized by its antimicrobial properties, which shall contribute to wound closure (9,10). The knowledge of the current scenario regarding the relevance of chitosan-based films in applied to wound dressing formulation development is essential to guide new studies related to the subject. With the constant raise of scientific publications of the main authors and periodicals on the subject of the last decades, this study aimed to establish a direct relation of plain chitosan films on wound closure rate based on recently studied data. In order to achieve this, the available literature was gathered after a specific search in Scopus database, followed by a bibliometric analysis. Different tools were used for document filtering and graphical representation of results. Results were found classified as acceptable or not acceptable based on wound closure metrics and statistical information presented by each paper. Limitations regarding the development of methodologies were observed. It is concluded that chitosan films show a positive correlation with wound closure rate in rats. However, no correlation with chitosan concentration in wound dressing films could be observed ($p>0.05$).

2. MATERIALS AND METHOD

2.1 Article Research

Research articles containing the term “chitosan films” in the title, keyword and abstract were collected using the Scopus database. More specifically, 81566 scientific articles were collected between 1934 and 2022. All review articles were later excluded from the search. The number of papers published per year was plotted using QtiPlot Software. Articles with publication year of 2021 and 2022 were excluded, as the final number of articles is not consolidated yet. The date of the retrieval was 12th July 2021.

2.2 Scientific scenarios evaluation with VOSviewer

All documents were sorted by citation. The bibliographical information for the documents containing abstract, author keyword and index keywords were exported as a RIS document and exported into VOSviewer (version 1.6.10) software. Different trending topics and themes were identified. From the scientific scenario, the search was refined with the terms “wound dressing” AND “wound closure”. This search produced 32 documents from 2003 to 2020.

2.3 Data Extraction

The articles were analyzed according the following criteria: chitosan concentration in the films, control group

number of replicates (greater than 3), standard deviation (i. e. Error bars) and *in vivo* wound closure rate measurement and follow-up times. Based on the inclusion criteria, information from all eligible publications were extracted. The following information were included in each study: name of first author, year of publication. Regarding *in vivo* wound closure rate (WCR) analysis, data were from plots curves. Engauge Digitizer 3.0 (by Mark Mitchell) software was used to extract data from figures for studies in which exact data were not shown in the text orlisted in tables. Data acquired with these methods were verified, and only those data with the same direction of effect as the original article were included. From data extracted, the 95% confidence intervals (CI95%) wascalculated.

2.4 Statistical Analysis and Meta-analysis

WCR for chitosan films and control samples was evaluated using mean values and CI95% of each study. Then, WCR mean values measured in different time intervals were ploted using QtiPlot Software, followed by linear regression analysis. R^2 adjusted and R were obtained for both chitosan films and control groups. Meta-analysis and Forest plot were obtained using Jamovi software, version 1.6.23.0. The analysis was carried out using the Fisher r- to-z transformed correlation coefficient as the outcome measure. A random-effects model was fitted to the data. Theamount of heterogeneity (i.e., tau^2), was estimated using the restricted maximum-likelihood estimator (11,12). In addition to the estimate of tau^2, the Q-test for heterogeneity and the I^2 statistic are reported. In case any amount of heterogeneity is detected (i.e., $tau^2 > 0$, regardless of the results of the Q-test), a prediction interval for the true outcomes is also provided (13). Studentized residuals and Cook's distances are used to examine whether studies may be outliers and/or influential in the context of the model. Studies with a studentized residual larger than the 100x (1 - 0.05/(2 X k))th percentile of a standard normal distribution are considered potential outliers (i.e., using a Bonferroni correction with two-sided alpha = 0.05 for k studies included in the meta-analysis). Studies with a Cook's distance larger than the median plus six times the interquartile range of the Cook's distances are considered to be influential. The rank correlation test and the regression test, using the standard error of the observed outcomes as predictor, are used to check for funnel plot asymmetry. The influence of chitosan concentration on WCR was accessed with one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

Skin wounds can be a common complication of several disorders, such as diabetes, and they can pose great burden to patient's health, seriously affecting their life quality and with possible evolution to amputation. The development of novel wound dressing with bioactive polymers, as chitosan, showed to be a hot topic at present. However, the direct relationship between the chitosan-based dressings and wound closure rate (WCR) is often neglected, which makes that of chitosan films are not fully explored in the development of novel formulations. Therefore, there is an urgent need for a systematic review and quantitative research on the relationship betweenthe chitosan films and WCR.

3.1 Article search

The searches in the database followed a criterions flow. The first generic search returned 81566 scientific publications in article format, of which initially were filtered, only those published from 1934 to 2020 were considered, thus remaining 7639 articles. The increased interest in this topic is evident from the distribution pattern of published articles, as shown in Figure 1.

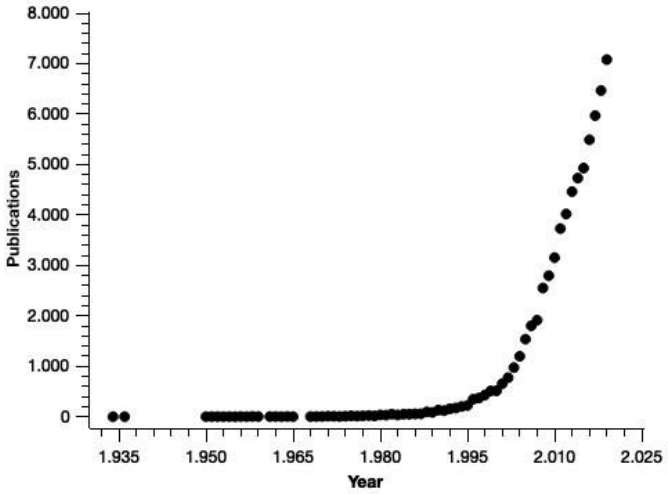

Figure 1. Article search related to “chitosan films” AND “wound healing” simultaneously sorted by year (Scopus search on 12th July 2021)

3.2 Scientific scenarios evaluation with VOSviewer

This search proved to be impractical and not objective, as it led to a too broad result. Thus, it was necessary to establish more criteria to narrow down the results. Given the objective of research in working only with publications that use a quantitative methodology to access WCR, so that it is possible to perform a meta-analysis, it was necessary to do add one more keyword. Such analysis was possible due to the identification of keywords clusters in the bibliographic map provided by VOSviewer software (version 1.6.16), as shown in Figure 2.

VOSviewer facilitated a broad understanding and interpretation of the scientific scenario and network patterns related to one theme. In this work, a network map of the trend topics according to the keywords used from 1934 to 2020, using keywords and abstract analysis. The size of the circles represents the frequency of appearance as the keywords. The distance between the two circles indicates their correlation. A total 289 items divided into four clusters were found.

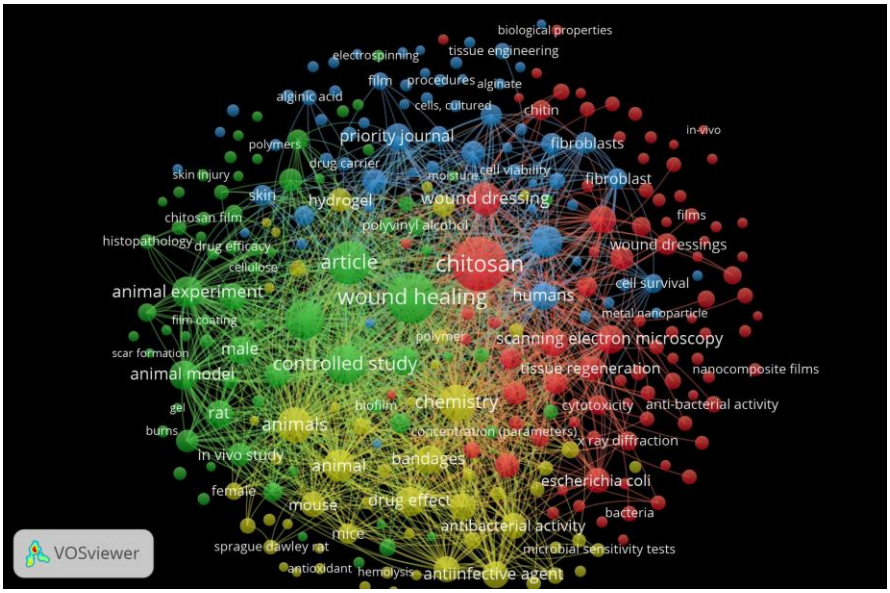

Figure 2: VOSviewer-enabled bibliometric analysis of clusters. Four clusters are shown in the map. The red cluster indicates physical-chemical material characterization and *in vitro* anti-bacterial activity. The blue cluster shows biological activities and biochemical markers features. The green cluster represents in vivo clinical studies using animal wound models. The yellow cluster is mainly related to different wound dressing bandages based on chitosan.

The density visualization (Figure 3) evidenced that several studies focused on in vivo animal models and controlled studies to access wound closure, wound contraction or re-epithelization have been highly mentioned. The densest themes indicated that such keywords would be effective choices to narrow down the search, i.e., among all papers, the papers whose titles, abstract and keywords contain “wound closure” should be read and investigated. In this context, a new search comprising the terms: “chitosan films” AND “wound healing” AND “wound closure" retrieved 32 articles (Table 1). These articles comprise the result of bibliometrics, which will be analyzed in the systematic review phase.

3.3 Data Extraction and analysis

From the bibliometric results, the systematic review phase was carried out in each of the 32 articles, following the criteria previously established in methodology section. This phase aimed to identify statistical data that are useful for meta-analysis, such as chitosan films composition, number of replicates, standard deviation or errorbars and the WCR metrics methodology.

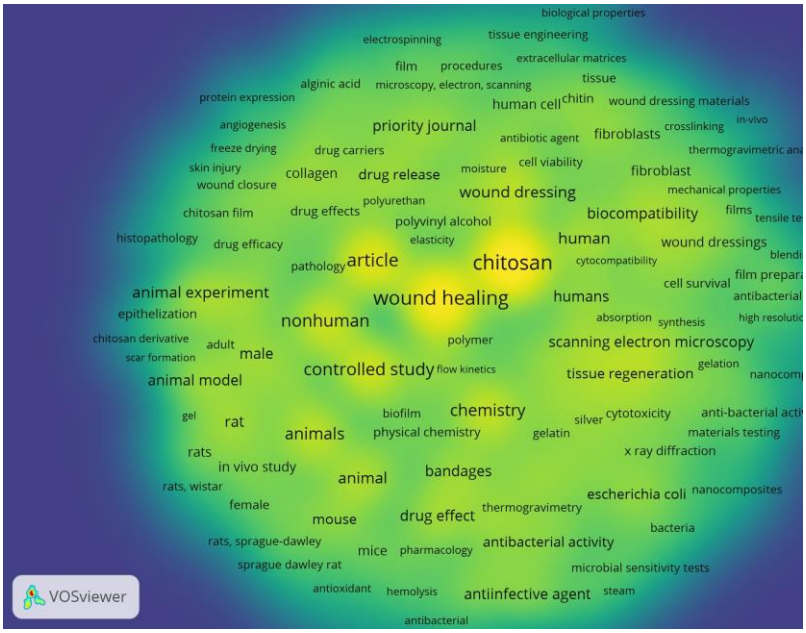

Figure 3. Density visualization map of the main keywords and terms in abstract, title or keywords

Table 1. Research articles retrieved from the optimized search in Scopus Database.

Result Number	Document title	Reference	Authors
1	Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing	(11)	Zhao, X., et al.
2	Chitosan film containing fucoidan as a wound dressing for dermal burn healing: Preparation and in vitro/in vivo evaluation	(12)	Sezer, A.D., et al.
3	Electrospun nanostructured chitosan-poly(vinyl alcohol) scaffolds: A biomimetic extracellular matrix as dermal substitute	(13)	Sundaramurthi, D., et al.
4	Diabetic wound regeneration using peptide-modified hydrogels to target re-epithelialization Open Access	(14)	Xiao, Y., et al.
5	Nitric oxide-releasing chitosan film for enhanced antibacterial and in vivo wound-healing efficacy	(15)	Kim, J.O., et al.
6	Copper metal-organic frameworks loaded on chitosan film for the efficient inhibition of bacteria and local infection therapy	(16)	Ren, X., et al.
7	A preliminary investigation of chitosan film as dressing for punch biopsy wounds in rats	(17)	Khan, T.A. and Peh, K.K.
8	Mechanical, structural and physical aspects of chitosan-based films as antimicrobial dressings	(18)	Escárcega-Galaz, A.A., et al.
9	Experimental study on effects of adipose-derived stem cell-seeded silk fibroin chitosan film on wound healing of a diabetic rat model	(19)	Wu, Y.-Y., et al.
10	Physical preparation of alginate/chitosan polyelectrolyte complexes for biomedical applications	(20)	Alsharabasy, A.M., et al.
11	Ionic Hydrogel Based on Chitosan Cross-Linked with 6-Phosphogluconic Trisodium Salt as a Drug Delivery System	(21)	Martínez-Martínez, M., et al.
12	Preparation and characterization of novel eggshell membrane-chitosan blend films for potential wound-care dressing: From waste to medicinal products	(22)	Li, X., et al.
13	Composite hydrogel formulations of stratifin to control MMP-1 expression in dermal fibroblasts	(23)	Rahmani-Neishaboor, E., et al.
14	Robotic laser tissue welding of sclera using chitosan films	(24)	Garcia, P., et al.
15	Erythropoietin/aloe vera-releasing wet-electrospun polyvinyl alcohol/chitosan sponge-like wound dressing: In vitro and in vivo studies	(25)	Naseri-Nosar, M.,et al.

16	Development and in vitro/in vivo evaluation of HPMC/ chitosan gel containing simvastatin loaded self-assembled nanomicelles as a potent wound healing agent	(26)	Varshosaz, J., et al.
17	Emerging trends in therapeutic algorithm of chronic wound healers: Recent advances in drug delivery systems, concepts-to-clinical application and future prospects	(27)	Shao, M., et al.
18	The single and synergistic effects of montmorillonite and curcumin-loaded chitosan microparticles incorporated onto poly(lactic acid) electrospun film on wound-healing	(28)	Naseri-Nosar, M., et al.
19	Synthesis of a Semi-Interpenetrating Polymer Network as a Bioactive Curcumin Film	(29)	Mayet, N., et al.
20	Layer-by-layer assembly of epidermal growth factors on polyurethane films for wound closure	(30)	Kulkarni, A., et al.
21	Effect of adipose derived nucleated cell fractions with chitosan biodegradable film on wound healing in rats	(31)	Mehrtash, M., et al.
22	Preparation, characterization and evaluation of a new film based on chitosan, arginine and gold nanoparticle derivatives for wound-healing efficacy	(32)	Wang, K., et al.

The first step, considering abstracts and keywords, aims at publications that have some statistical data. This phase allowed the selection of 4 articles that met the criteria, with 208 publications being disregarded. Paper 5,6, 23 e 27 published by Kim et. al., 2016, Ren et al., 2019, Pensara et al., 2020 and Pereira et al., 2020, respectively, were selected (Table 2).

Table 2. Studies included in Bibliometric and Meta-analysis.

Authors	Result Number	Chitosan%	Replicates	WCR (%) metrics	Measurements (days)
Kim et al., (2015)	5	1%	8	(A0 - At/A0)*100	3, 6, 9, 12 and 15
Ren et al., (2019)	6	2%	6	(A0 - At/A0)*100	3, 7, 14 and 21
Pensara etal., (2020)	23	1.5%	6	(A0 - At/A0)*100	3,5,7,14 and 21
Pereira etal., (2020)	27	1%	15	(A0 - At/A0)*100	2,5,7,10 and 14

This study evaluated the influence of chitosan films on WCR% measured as $(A_0 - A_t/A_0) *100$, where A_0 means wound area at day 0 and A_t means the wound area at time t. The linear correlation results obtained for the selected articles is shown in Table 3. For all articles studied, the WCR showed a positive correlation with chitosan Films. Moreover, chitosan concentration in the wound dressing formulations was expected to have an influence in these results, due to the higher correlation (R adjusted) and coefficient of determination (R^2 adjusted) observed for the work of Ren et al., (2019), which used 2% wt of chitosan to produce chitosan films as wound dressing material.

Table 3. Research articles considered and the results from linear regression analysis.

Chitosan Films				
Authors	**N**	**Total replicates**	**R^2 adjust**	**R adjust**
Kim et al., (2015)	8	40	0.9485	0.9739096467
Ren et a., (2019)	6	24	0.9933	0.9966443699
Pansara et al., (2020)	6	30	0.9791	0.9894948206
Pereira et al., (2020)	15	75	0.9691	0.9844287684
Control Group				
Authors	**N**	**Total replicates**	**R^2 adjust**	**R adjust**
Kim et al., (2015)	8	40	0.8633	0.9291393867
Ren et a., (2019)	6	24	0.8769	0.9364293887
Pansara et al., (2020)	6	30	0.8712	0.9333809512
Pereira et al., (2020)	15	75	0.7719	0.8785783972

However, no statistically significant differences were observed for the results obtained from chitosan films with 1, 1,5 or 2% ($p>0.05$). Such fact might be explained by the fact that the studies produced chitosan films using different solvents, chitosan grades, Molecular weight and deacetylation degrees. Additionally, Kim et al (2015) and Pansara et al., (2020), used different concentrations of glicerol as a plasticizer for the obtention of chitosan films. Itis possible that such unstandardized parameters had led to variable results, hindering their comparison.

3.4 Statistical Analysis and Meta-analysis

3.4.1 Chitosan Films

The results in Figure 4 show the meta-analysis results for Chitosan films. In a random-effects (RE) model, the true effect is presumed to vary from study to study, and included in true heterogeneity test (46). The RE model estimated effect size by considering the primary study a random factor (47). A total of k=4 studies were included in the analysis for Chitosan Films. Moreover, none of the results reached the null effect line which means the chitosan films effect on the results is favored and the null value does not lie within the confidence interval and the results are statically significant.

The observed Fisher r-to-z transformed correlation coefficients ranged from 1.9257 to 2.5713, with all estimates being positive (100%). The estimated average Fisher r-to-z transformed correlation coefficient based on the random-effects model = 2.362 (95% CI: 1.954 to 2.771). Therefore, the average outcome differed significantly from zero ($z = 11.3291$, $p < 0.0001$), as shown in Table 4 (Tau^2 Estimator: Restricted Maximum-Likelihood (11)).

Table 4. Random Effects Model Parameters for Chitosan Films.

	Estimate	**Standard Error**	**Z**	**p**	**CI Lower Bound**	**CI Upper Bound**
Intercept	2,362	0. 209	11.3	<.001	1,954	2,771

The presence of true heterogeneity between studies was identified by the Q test. According to the Q-test, there was

no significant amount of heterogeneity in the true outcomes (Q(3) = 1.4996, p = 0.6824, tau² = 0.0000, I² = 0.0000%). These results are summarized in Table 5. For quantification of the heterogeneity, I² index was used (48), which describes the proportion of total variation across the studies that is due to heterogeneity. I²-values greater than 50% were assumed to indicate substantial heterogeneity and meta-regression was carried out as an attempt to explore the source of the heterogeneity. This meta-analysis resulted in I² = 0% (Table 5), which means that no significant heterogeneity can be found (p < 0.001) (49).

Table 5. Heterogeneity Statistics.

Tau	Tau²	I²	H²	R²	df	Q	p
0. 000	0 (SE=0.1514)	0%	1. 000	-	3. 000	1. 500	0. 682

It is observed in the Forest Plot (Figure 4) four horizontal lines, a rectangle in each of them, a vertical dashed line, and a diamond-shaped symbol. The horizontal lines measure the maximum and minimum ranges of each study, the rectangles represent the contribution of each of these findings, i.e. the size of population, the vertical dashed line indicates the null effect limit, on the right side are the positive values and on the left to the negatives, and the diamond shows the effect size, that is, the general result of the RE model (50). An examination of the studentized residuals revealed that none of the studies had a value larger than± 2.4977 and hence there was no indication of outliers in the context of this model. According to the Cook's distances, none of the studies could be considered to be overly influential.

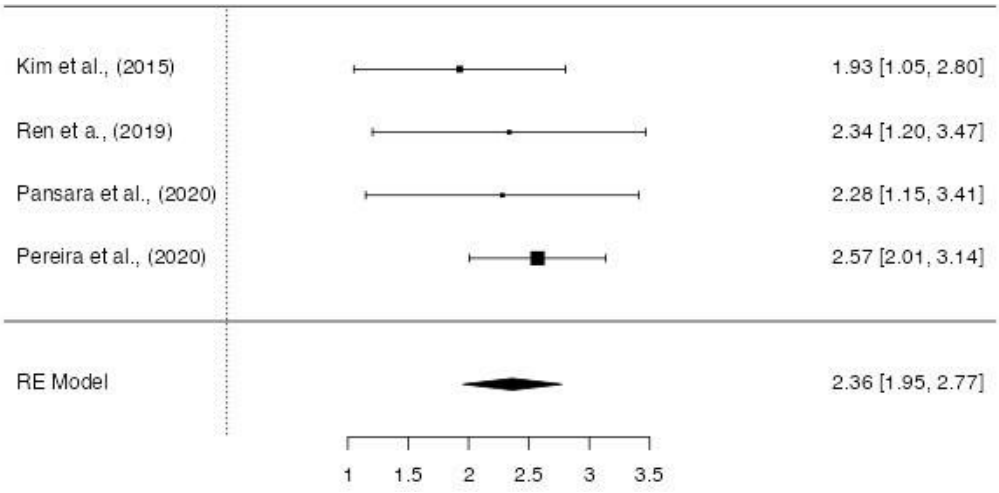

Figure 4: Forest plot of the random-effects models of the effect chitosan films vs. wound closure rate on in vivo animal models.

When considering the weight of each study in the meta-analysis, it was observed that most of them feature very similar weights, which shows that they can be considered significant for the result of the effect size. It is also evident from Figure 4 that all studies show a positive correlation. Thus, the meta-analysis showed that chitosanfilms and wound healing are related, in a way that chitosan-based wound dressings influence highly and positivelyin wound closure rate.

Publication bias is represented by the Funnel Plot, which shows the tendency for the results to be systematically different from real effect, represented by the doted central line (51). It is a figure plotted on the Cartesian graph where the x-axis shows the Fisher's z Transformed Correlation Coefficient and the y axis shows the Standard Error. Studies with greater variability appear at the top of the graph and around the mean and the wider part of the funnel indicates less accurate studies (46). Studies that lie outside the funnel indicate results that are very different from those found (12). Figure 5 shows the funnel graph for Chitosan films.

Neither the rank correlation nor the regression test indicated any funnel plot asymmetry (p = 0.7180 and p = 0.4561, respectively). Such results indicate that the studies do not show great variability, as they are located at the middle of the funnel,as well as none of them is located outside the funnel, which evidences the absence of publication bias.

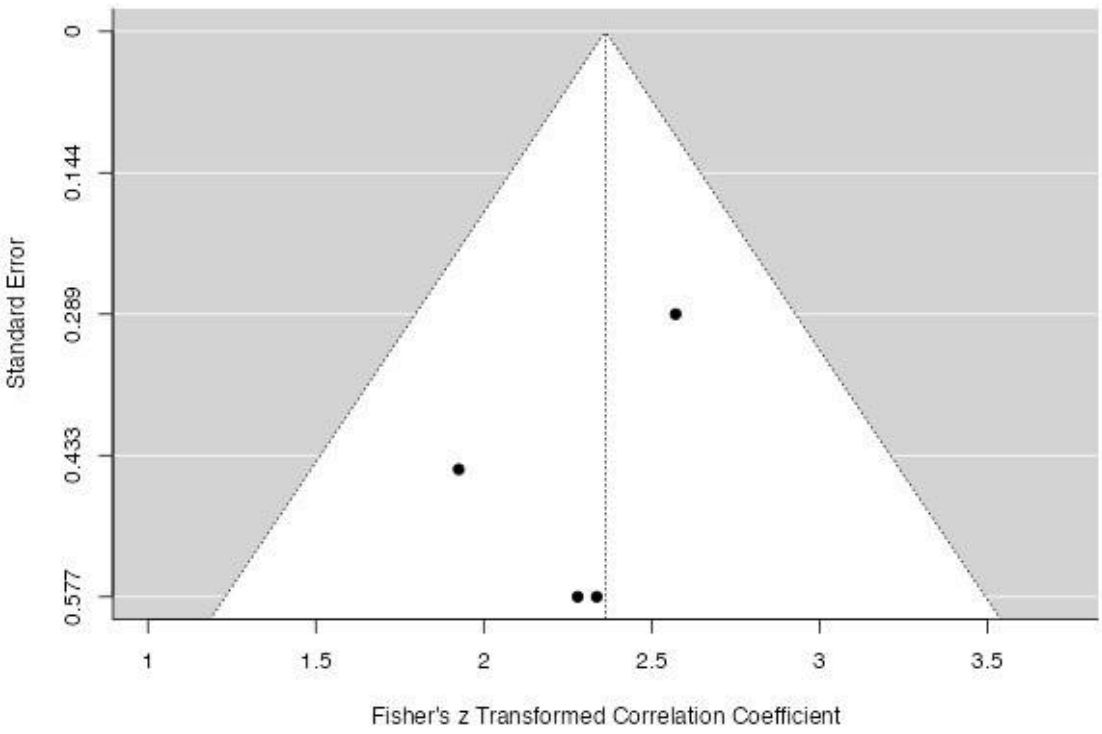

Figure 5. Funnel Plot for Chitosan Films.

3.4.2 Control groups

A total of k=4 studies were included in the analysis of Control groups, as in the previous section. The observed Fisher r-to-z transformed correlation coefficients ranged from 1.3695 to 2.3363, with the majority of estimates being positive (100%). In this context, the correlation between control groups with WCR% show a positive relation. The estimated average Fisher r-to-z transformed correlation coefficient based on the random-effects model was = 1.7692 (95% CI: 1.354 to 2.185). Therefore, the average outcome differed significantly from zero (z = 8.3458, p < 0.0001). Such results are summarized in Table 6.

Table 6. Random-Effects Model Parameters for Control Groups.

	Estimate	Standard Error	Z	P	CI Lower Bound	CI Upper Bound
Intercept	1.77	212	8.35	<.001	1,354	2,185

The heterogeneity results are shown in Table 7. The Q test constitute one of the most used method to assess the heterogeneity and assumes that the findings of primary studies are the same (null hypothesis) and checks whether the data found refute the null hypothesis (48). If the null hypothesis is confirmed, the studies are considered homogeneous (p > 0.05). The magnitude of heterogeneity is verified mainly by calculating the I^2, which ranges from 0 to 100%. I^2 is obtained from the Q value and the number of studies involved in meta-analysis. An I^2 greater than 50% indicates substantial heterogeneity and, above 75%, considerable heterogeneity. The greater the heterogeneity, the greater the questioning about the validity of combining results. According to the results from Table 7, the true outcomes appear to be heterogeneous (Q(3) = 22.8532, p < 0.0001, Tau^2 = 0.1492, I^2 = 84.2838%).

Table 7. Heterogeneity results for Control Groups

Tau	Tau²	I²	H²	R²	df	Q	p
0. 386	0. 1492 (SE = 0. 1468)	84.28%	6. 363	.	3. 000	22,853	<.001

The diamond symbol in Forest plot (Figure 6) represents the combined mean of all effects from the comparison studies analyzed by the meta-analysis. Moreover, the analysis of Figure 6 showed a 95% prediction interval for the true outcomes is given by 0.9056 to 2.6329. Hence, even though there may be some heterogeneity, the true outcomes of the studies are generally in the same direction as the estimated average outcome.

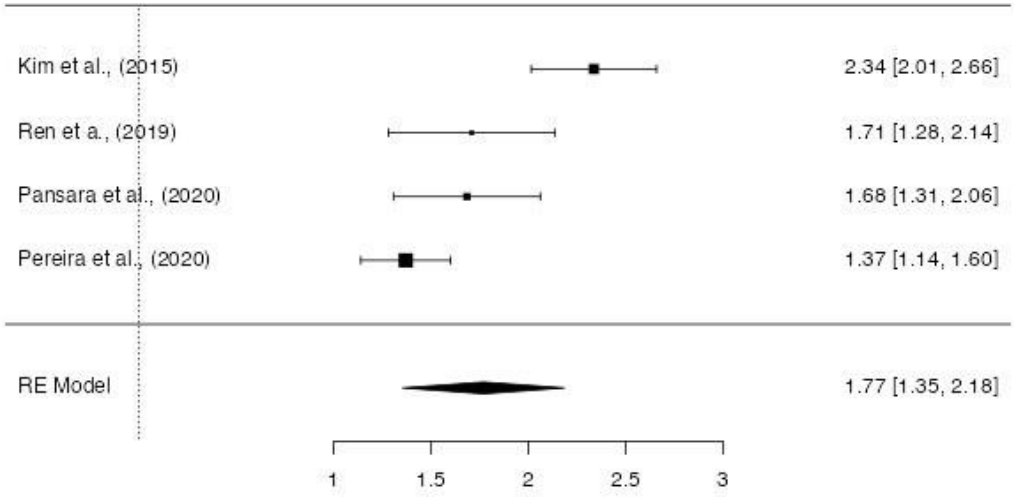

Figure 6. Forest Plot for Control Groups

An examination of the studentized residuals revealed that one study (Kim et al., (2015)) had a CI95% value larger than ± 2.4977 and may be a potential outlier in the context of this model. However, an outlying case may not so harmful to the analysis if it exerts little influence on the results. In this context, the exclusion of a study from the analysis leads to no considerable changes in the fitted model. Cook's distance is a parameter that predicts the extension of effect would be caused by the deletion of one study and its influence on the fitted values of all 4 studies simultaneously. According to the Cook's distances, none of the studies could be considered to be overly influential.

In systematic reviews, the presence of this bias can be identified using a funnel graph and statistical tests. The use of these techniques is recommended for meta-analyses with ten studies or more and is based on matters of estimation and accuracy. The inaccurate studies, generally carried out with small size samples may find positive or negative results (statistically significant or not) due to the influence of chance. They would be symmetrically distributed in the widest part of the funnel. Higher precision studies, generally in smaller numbers, would be closer to the real value and located in the narrowest part of the funnel. Funnel plot is shown in Figure 7. Neither the rank correlation nor the regression test indicated any funnel plot asymmetry (p = 0.7500 and p = 0.6928, respectively).

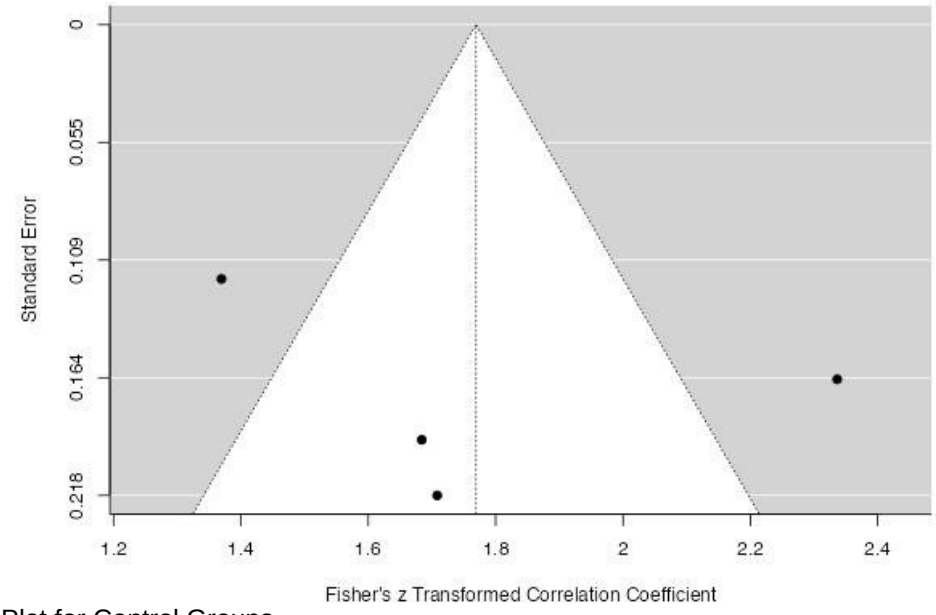

Figure 7. Funnel Plot for Control Groups

CONCLUSIONS

This paper describes systematic review and meta-analysis over the subject of chitosan-based wound dressings. The search for scientific papers was standardized and was evaluated according the most frequent terms, which led to a narrower search result. All 32 studies were systematically analyzed and sorted according to important data to evaluate the impact of chitosan films on in vivo wound closure rate. The data extraction from previous studies published in literature revealed a good homogeneity of a positive relationship between the rate of wound healing and the use of chitosan-based wound dressings.

The results of this work shows that further research and clinical evaluations of chitosan films on wound healing rate are still nedded, and it might help to understand the relationship between the actual contribution of chitosan and chitosan concentration and in vivo wound healing evaluation.

Our results showed that chitosan films are potential biomedical materials that might provide a reference to new approaches for the treatment chronic wounds. The advantages of this study include the exploration ofstatistical and meta-analysis tools, with important parameters as heterogeneity, variation analysis and others were addressed.

Sample CRediT author statement

Thaís Nogueira Barradas: Conceptualization, Methodology, Data analysis, and Writing-Original draft preparation.

Fernando G. de Souza Junior: Conceptualization, Supervision, Reviewing and Editing.

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