Exploring the Potential of Bovine Colostrum as a Bioactive Agent in Human Tissue Regeneration: A Comprehensive Analysis of Mechanisms of Action and Challenges to be Overcome

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March 08, 2024

Abstract

Bovine colostrum is a rich source of bioactive compounds, including growth factors, that may promote tissue regeneration. Studies suggest it has potential therapeutic benefits in humans, but further research is needed to fully understand its mechanisms of action. This study aims to review scientific articles on the use of bovine colostrum as a bioactive agent for tissue regeneration in humans and twelve articles were selected. In vitro studies show that bovine colostrum can benefit cells by reducing cell proliferation and inducing differentiation. The concentration of colostrum used has a direct impact on the results, with no positive results observed at some high concentrations (20%). Extracted portions of colostrum (EVs and IMF) showed better results than the complete colostrum. In vivo studies show that bovine colostrum is a promising natural source for wound healing, as its bioactive factors help in the orderly progression of the healing process. However, some studies did not find any benefit related to tissue regeneration, and more research is needed to explore the full potential of colostrum in regenerative medicine and to develop more effective therapies for wound healing.

INTRODUCTION

Wound healing involves the replacement of damaged tissue with newly formed tissue and occurs in four phases: hemostasis, inflammation, proliferation, and remodeling. The transition from the inflammatory to the proliferative phase is a crucial step in wound healing, typically occurring around day 4 (KIM et al., 2022; LANDÉN, LI & STAHLE, 2016; ZOMER & ANDREA, 2018). This process is regulated by various cell types, enzymes, cytokines, and growth factors (DONG & GUO, 2021; KAUR; JOSHI; SINGH, 2022).

Bioactive agents used in tissue regeneration include growth factors like epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), and platelet-derived growth factor (PDGF), which stimulate cell proliferation, angiogenesis, extracellular matrix synthesis, and cell differentiation. Additionally, substances such as antioxidants, polyphenols, and omega-3 fatty acids have been studied for their effects on tissue regeneration, including reducing oxidative stress and modulating inflammation. The combined use of these bioactive agents can increase the efficacy of tissue regeneration and promote faster and more complete recovery (TANIDEH, et al., 2017; JONES et al., 2019)

Bovine colostrum is a nutrient-rich milk secretion containing immunoglobulins, growth factors, and other bioactive compounds that support calf nutrition and immune development. Studies have suggested that bovine colostrum may also benefit human health, such as improved immune function, reduced risk of respiratory and gastrointestinal infections, and enhanced gut health (SANGILD et al., 2021; GHOSH & IACUCCI, 2021; MEHRA et al., 2022; LINEHAN, ROSS & STANTON, 2023).

Bovine colostrum has been used as a nutritional and therapeutic supplement in various clinical conditions in humans, such as malnutrition, infections, inflammatory bowel disease, and musculoskeletal injuries (PLAY-

FORD et al., et al., 2021). However, further studies are necessary to fully understand the mechanisms by which bovine colostrum exerts its beneficials effects on human health. This study aims to review scientific articles on the use of bovine colostrum as a bioactive agent for tissue regeneration in humans, covering topics such as its nutritional and immunological properties, ability to promote cellular and tissue regeneration, and potential mechanisms involved. The goal is to provide an updated overview of the therapeutic potential of topical use of bovine colostrum in tissue regeneration and its clinical implications.

METHODS

For the evaluation of cellular and immunological components, a systematic review was performed according to the guidelines provided in the PRISMA-P (MOHER et al., 2015) statement to answer the following question: "Does the application of bovine colostrum help tissue healing of wounds?". To group relevant studies in the area, it was carried out by two qualified researchers. The search was carried out in the following databases: Web Of Science, Medline (through doing Pub Med), Scopus, Embase and Chochrane. We took into consideration both in vivo and in vitro studies, including articles written in English.

To conduct the research, the keywords "bovine colostrum," "wound healing," and "skin" were used. Duplicate articles were removed, and all relevant information was stored in an online account maintained by the principal investigator. We evaluated all studies for eligibility based on our acronym-based inclusion and exclusion criteria for Patient, Intervention, Comparison, and Outcomes (PICO). For patients, we included studies related to tissue regeneration with the search terms "Wound healing," "skin," "healing wound," "healings wound," and "wound healings" OR "guided tissue regeneration" and "colostrum" OR "colostrums" for intervention. For intervention, we included studies that used some form of administration of bovine colostrum. We only considered articles that had relevant terms related to bovine colostrum and tissue regeneration in their title or abstract, and review articles were excluded (Figure 1).

RESULTS

Twelve articles related to bovine colostrum on tissue regeneration were included and the table below provides a comprehensive overview of the studies found in our search that investigate the effects of colostrum in various cell types and animal models. The table comprises details such as author and publication year, study type (in vitro or in vivo), utilized cell or animal model, colostrum concentration, collection time, and each obtained result. These studies provide valuable information about the potential benefits of colostrum, particularly when collected in the early stages of lactation.

Table 1 – Selected articles in systematic review.

Autor/ publication yerar	**Type**	**Cellular type or animal model**
Klagsbrun 1980.	In vitro	madin-darby canine kidney epithelial cells (M
Takayama, Kitsunai & Mizumachi. 2001.	In vitro	WI-38 fibroblasts
Kovacs et al. 2009.	In vitro	human keratinocytes (HaCaT)
Doillon et al. 2011.	In vitro	human foreskins fibroblasts
Kshirsagar et al. 2015	In vivo	humam patient with deep wounds (stage II-IV
Tanideh et al. 2017. DOI:10.1007/s00580-016-2347-5	in vivo	sprague Dawley rats
Kovacs et al. 2020.	In vitro	normal human keratinocytes (NHKs) and nor
Seo et al. 2021.	In vitro	cell line HaCaT
Çeleb et al. 2022.	In vivo	male wistar albino rats
Kim et al. 2022.	In vivo and in vitro	NIH-3T3 (murine fibroblast cell line) SVEC4-
Tarff et al. 2022.	In vivo	C57BL/6 mice
Kaur et al. 2022.	In vitro and in vivo	Cell NIH3T3, wistar albino rats

DISCUSSION

In vitro

Fibroblasts

Evaluating the activity of colostrum in fibroblasts is highly valuable since fibroblasts are essential during inflammation, cell proliferation, and differentiation, and are responsible for deposition of extracellular matrix (ECM) components, wound contraction, and new ECM remodeling (GOMES et al., 2021; DESJARDINS-PARK, FOSTER & LONGAKER, 2018). In our search, the oldest work that used colostrum in this cell type was that of KLAGSBRUN (1980) who used colostrum to complement the culture medium in the growth of human fibroblasts and in four other cell lineages: Madin-Darby canine kidney epithelial cells (MDCK), rabbit kidney epithelial cells (RK13), primary calf kidney cells (CK) and primary bovine embryonic kidney cells (BEK).

Contrary to expectations, sparse cultures of human fibroblasts and mouse fibroblasts were unable to grow in DMEM supplemented with colostrum. The ideal colostrum concentration for MDCK growth was 2.5% (vol/vol). At higher colostrum concentrations, adhesion of MDCK cells to the culture flask was poor and resulted in a reduced final saturation density. Although the colostrum prevented the growth of fibroblasts, it was not toxic because the growth of these cells resumed when the first day's colostrum was replaced with serum. The conclusion reached at the time was that colostrum contains specific factors necessary for epithelial growth, but lacks those necessary for the growth of fibroblasts KLAGSBRUN (1980). These findings suggest that colostrum may have promising effects on the growth of epithelial cells but might not be suitable for the growth of fibroblasts.

Another fibroblast study was performed in 2011 with human foreskin-derived fibroblasts, where they did not use powdered colostrum, but a complex composite fraction (IMf) of colostrum-derived serum. This fraction was used because, according to the authors, casein found in colostrum may end up masking the effect of bioactive compounds, such as insulin-like growth factor type 1 (IGF-1), and for processing the fraction, caseins were removed (DOILLON et al., 2011). Takayama et al. (2001) also used casein-free colostrum fractions. Other studies report that not only caseins, but also their peptides inhibit cell proliferation and stimulate differentiation, even if they do not show toxic results in toxicity tests (LEDESMA-MARTÍNEZ et al., 2019; LONETTI et al., 2019).

Casein is a protein present in milk and its derivatives that has the ability to inhibit cell proliferation through various mechanisms. The protein can be broken down into peptide fractions, including casomorphin, which has inhibitory effects on cell proliferation. In addition, casein can bind to specific receptors on cells, such as estrogen receptors, and inhibit their activation, decreasing the production of cell growth factors such as epidermal growth factor (EGF). Casein can also affect the activity of enzymes involved in cell proliferation, such as cyclin-dependent kinases, interrupting cell cycle progression and consequently, cell proliferation (MINEO et al., 2018; TANG et al., 2021)

Still regarding the work carried out by Doillon et al., (2011), at the lowest IMf dilution (1:0·5) the activity of fibroblasts in the collagen gel was close to the control, while higher concentrations (1:10–1 :1) induced an increase in fibroblasts and stress fiber formation that was observed mainly in fibroblasts cultured in 5% FBS and with high dilutions of IMf. In general, fibroblasts had better activity at higher concentrations of IMf, while those exposed to low dilutions of IMf exhibited widely aggregated and elongated F-actin (DOILLON et al., 2011).

F-actin plays a crucial role in tissue regeneration by supporting cell adhesion, migration, and extension, which are essential processes for successful tissue repair. During tissue repair, F-actin undergoes dynamic changes to promote cellular movement and the formation of new tissue. For example, in response to tissue damage, F-actin helps to form a contractile ring that allows cells to divide and proliferate, which is important for tissue regeneration (YADAV et al., 2019). Additionally, F-actin helps to direct the migration of cells towards the site of injury and provides mechanical support to maintain cell shape during tissue remodeling (CHANG et al., 2020). Overall, F-actin is a critical component of the cellular machinery involved in tissue regeneration and repair.

Takayama et al., (2001) investigated the effects of bovine colostrum serum (final concentration 1%) on the

migration of W138 human fibroblasts and verified that when added to DMEM, it increased the contraction of the collagen gel, with effects similar to those of TGF -ß (10ng/ml) (control) in the first 12 hours of contraction, however, cell growth promotion was not observed. The test carried out evaluating the contraction of the collagen gel is a classic tool to study cells of the extracellular matrix, where the cell-laden collagen is placed in a container and forms a gel adhered to the bottom. From there, the diameter of the gel surface area is used as a parameter to quantify the level of cell contractility (ZHANG et al., 2019a).

Recently, in 2022, KIM et al., tested the ability to promote wound repair using extracellular vesicles (EVs) from milk and bovine colostrum, which, according to the authors, provide a cocktail of anti-inflammatory cytokines and factors to store the remodeling of the destroyed extracellular matrix. These vesicles are secreted by most cell types and play an important role in cell communication. Bovine milk is an accessible source of them, which can be obtained easily and in large quantities by serial centrifugation and filtration steps (HAN et al, 2022; ZHANG et al., 2019b).

They verify higher concentrations of EVs were present in biological fluids (serum or milk) compared to cell culture medium (CCM). The yield of EVs from colostrum was about 60 times higher than that from CCM. Then, they investigated the potential activity of colostrum EVs in the proliferation phase of wound healing. The colostro EVs treatment caused an approximately fourfold increase in scratch closure too (KIM et al., 2022).

The EVs of colostrum are small liposomes that transport various bioactive molecules, including growth factors and cytokines, which can play an important role in tissue regeneration (MECOCCI et al., 2022). The previous study demonstrated the ability of EVs to promote cell proliferation, migration, and differentiation of cells involved in wound healing and tissue regeneration (KIM et al., 2022). These properties make colostrum EVs a potential promising therapy for the treatment of wounds and other tissue regeneration conditions.

Based on the results of the studies found, it is evident that more research is needed to prove the effectiveness of bovine colostrum on fibroblasts. Although the study conducted by Klagsbrun (1980) produced unsatisfactory results in one of the cell types, Doillon et al. (2011) and Kim et al. (2022) obtained promising results. It is important to note that the positive results were found in studies that used specific portions of bovine colostrum, suggesting that although these portions show positive results in fibroblasts, when combined with other components of colostrum, they may be ineffective. Therefore, more research is needed to identify and understand the specific factors present in bovine colostrum that affect the activity of fibroblasts.

Keratinocytes

Keratinocytes are the most abundant cells in the epidermis and play a critical role in tissue regeneration. During the process of tissue regeneration, keratinocytes proliferate and migrate to the injured area to cover the wound and restore the cutaneous barrier (WANG et al., 2021; ZHAO et al., 2018). Additionally, these cells are capable of secreting growth factors, such as epidermal growth factor (EGF), which stimulate the proliferation and differentiation of other cells, including fibroblasts and endothelial cells, as well as the production of collagen and other components of the extracellular matrix (ECM). Recent research has explored the use of keratinocytes in tissue regeneration, either through autologous cell transplantation or mesenchymal stem cell therapy (WANG et al., 2021; HEYDARI et al., 2022).

Kovacs et al. (2009) investigated the effects of bovine colostrum on keratinocytes using HaCaT cells. Cell growth was assessed with varying colostrum concentrations (0.5-20%). The results showed that treatment with 5% and 10% colostrum for 24 hours stimulated keratinocyte proliferation ($p<0.01$), while lower concentrations (0.5%, 1%, and 2%) did not have significant effects, and the highest concentration (20%) had an inhibitory effect after 48 hours.

With the aim of increase the results, they analyzed the growth promoting effect induced by colostrum in immunofluorescence analysis, with the concentrations 5% and 10%, during 24h. The results show that colostrum induced increase in the number of cells. Then, they evaluated whether colostrum affect the cell migration, and to this, they kept HaCat in medium containing colostrum and observed the started to cover

a scrat área within 6h compared to with the presence of cells more elongated, typical of a migratory stage and after 24h the colostrum and FBS contributed to cell migration and also for proliferation, as expected from the results obtained in growth promoting activity.

The same author and collaborators published a new and more comprehensive article in 2020, using lower concentrations of bovine colostrum (KOVACS et al., 2020). In their first study, the tested concentrations had a wider range, from 0.5% to 20% (KOVACS et al., 2009), while in the second study, the concentrations ranged from 0.05% to 0.5% (KOVACS et al., 2020).

The study evaluated the effects of colostrum on human primary keratinocytes. Colostrum concentrations of 0.3% and 0.5% decreased the number of active phase keratinocytes and induced the expression of cyclin-dependent kinase inhibitors. Colostrum induced differentiation in keratinocytes, as demonstrated by morphological changes and increased expression of K16, an early marker of keratinocyte differentiation. Colostrum also increased the expression of late differentiation markers, including involucrin and filaggrin, and facilitated the transition from early to late stages of differentiation. These effects were observed in a dose-dependent manner (KOVACS et al., 2020). Filaggrin is an important protein in the formation of the skin barrier and its expression is associated with keratinocyte differentiation and formation of the stratum corneum, the protective layer of the skin (IRVINE, MCLEAN & LEUNG, 2011).

Using the 3D cell culture system with polystyrene scaffolds, the authors were able to observe that bovine colostrum supported early differentiation state by stimulating the expression of K1 in the suprabasal layers, improving the terminal differentiation process by promoting the stratification of keratinocytes and the expression of involucrin in the outermost layers. The study also investigated the role of calcium in the differentiation process of keratinocytes. The results showed that the addition of bovine colostrum had more pronounced differentiation characteristics than the addition of calcium to the culture médium (KOVACS et al., 2020).

Bovine colostrum also increased the levels of phospho-PI3K p85(Tyr458), phospho-Akt (Ser473), PLC-γ1 protein, and phospho-PKCα, indicating a possible relationship between the signaling pathways activated by bovine colostrum and the differentiation of keratinocytes. Additionally, the study observed that tyrosine kinase receptors (RTKs) and G protein-coupled receptors (GPCRs) appear to play an important role in the initial phase of keratinocyte differentiation. Activation of the epidermal growth factor receptor (EGFR) was also verified due to its involvement in promoting terminal differentiation through the PLC-γ1/PKCα pathway (KOVACS et al., 2020).

Other study, investigated the relationship between colostrum serum and the expression of AQP-3 using HaCaT cells. The researchers verified the cytotoxicity of colostrum in the cells and found that no concentration was cytotoxic. The cells increased their viability, especially at the highest concentration. RT-PCR was used to identify the AQP subtypes expressed in HaCaT, where AQP-3 and AQP-10 were identified as being expressed, with AQP-3 being the most abundant (SEO et al., 2021).

They then evaluated whether colostrum serum altered the expression of AQP-3 at concentrations of 1, 10, or 100 µg/mL. Cells treated with whey colostrum did not show a significant increase in expression compared to the control, while cells treated with fermented colostrum serum showed an increase in AQP-3 expression at concentrations of 10 and 100 µg/mL. The duration of AQP-3 expression with fermented colostrum at a concentration of 100 µg/mL was evaluated, and an increase in expression was observed at hour 2, with a peak at hour 3 and a decrease after hour 6. Hour 3 was then selected to identify the expression of AQP-3 at the protein level using fermented colostrum at concentrations of 0, 1, 10, and 100 µg/mL (SEO et al., 2021).

The authors found an increase in AQP-3 protein expression levels after treatment with fermented colostrum at concentrations of 10 and 100 µg/mL, with expression levels being 10.5 and 10.9 times higher than those of the negative control (SEO et al., 2021).. This suggests that fermented colostrum is positively related to AQP-3 expression in keratinocytes. Although there is no direct evidence in this study, it is possible that fermented colostrum regulates HaCaT keratinocyte proliferation by positively regulating AQP-3

The EGF, which is abundantly present in colostrum, is a known upstream regulator of AQP-3 expression in cancer cell cultures (LI et al., 2013; SMITH et al., 2023). Additionally, lactoferrin, exopolysaccharides, and bioactive peptides with low molecular weight are present in fermented milk and serum, which can stimulate the proliferation and healing of skin cells. Previous studies have reported an increase in keratinocyte proliferation and AQP-3 expression after treatment with <3 kDa molecular weight compounds produced during cheese fermentation (LÖNNERDAL, 2010). These findings suggest that low molecular weight peptides produced during fermentation activate keratinocytes.

The studies found in this research related to the use of bovine colostrum in keratinotics bring not only physiological data, but there are also works that deepened the analysis and, as a result, obtained promising results in relation to the expression of proteins and other growth factors that are related to the process of tissue regeneration. In general, *in vitro* studies show that bovine colostrum may have a beneficial effect on cells, both with the use of fibroblasts and keratinocytes. This mainly involves stimuli that decrease cell proliferation and induce differentiation. It is worth noting that the concentration of colostrum used has a direct impact on the results, and extracted portions appear to yield better results than the complete colostrum

IN VIVO

In our search, the in vitro studies found included studies in Sprague Dawley rats (TANIDEH et al. 2017), Balb/c mice (KIM et al., 2022), female guinea pigs (DOILLON et al., 2011); wistar albino rats (ÇELEBI et al., 2022; KAUR et al., 2022), female and male healthy C57BL/6 mice (TARFF et al., 2022) and humans (KSHIRSAGAR et al., 2015)

Kshirsagar et al. (2015) compared the use of colostrum dressing versus conventional dressing in patients aged 20 to 60 with stage II to IV deep wounds. The experimental group received the application of powdered colostrum twice a day, at 7am and 7pm, for 15 days, with wound observations on the 3rd, 6th, 9th, 12th, and 15th day. The dependent variables were the healing process, wound size, wound impregnation, amount and color of exudate, pain felt by the patient, and reduction in wound stages during the days of application. The conventional dressing was done with betadine and hydrogen peroxide. The study analyzed the effect of powdered colostrum by comparing the dependent variables in the experimental and control groups. The study found that the use of colostrum dressing yielded better results, with only 20% of patients maintaining their lesions in weeks 3 and 4, compared to 40% of patients in the conventional dressing group. Between the 12th and 25th day, only 15 patients still needed colostrum dressings, as opposed to 45 patients who needed conventional dressing. The results also showed that colostrum dressing can reduce hospitalization time, promote ulcer healing, and reduce pain in cases of deep ulcers. Colostrum dressing is an economical, readily available, non-immunogenic, and easy-to-apply option.

Kim et al., 2022 tested the Colos EVs, in wounds created in BALB/c mice and histological analysis performed 13 days after wounding showed that both groups treated with Colos EVs showed a thicker dermal layer and had enhanced re-epithelialization, with more granulation tissue compared to the group without colostrum. In this work, treatments with Colos EVs induced elastin deposition and CD31 expression, which are indicative of increased angiogenesis. In addition, the application of Colos EVs seemed to accelerate wound healing in a concentration-dependent manner, where the higher the concentration, the faster the wound healing was, even faster than the control used with the epidermal growth factor human recombinant (rhEGF, Nepidermin), which is commonly used as a drug to promote skin wound healing.

The other study that used colostrum fraction was conducted by DOILLON et al. (2011), where they treated wounds with a complex composite fraction of colostrum and compared two different models, one treated immediately and the other treated once closed. Results from the first model demonstrated that treatment with IMf (1:0·5) for 23 days led to less wound contraction when compared to treatment with PBS. However, there was no significant difference in wound contraction when treated with different dilutions of IMf (ranging from 1:1000 to 1:10), except for a significant decrease in wound contraction on day 14 at 1:1 dilution of IMf and on day 23 at the lowest dilution (1:0·5) compared to control wounds. On day 23, histological sections

showed that the areas occupied by the wound tissues were significantly higher after treatment with 1:1 IMf compared to control wounds, but not with other conditions.

The second model showed that wounds treated with cIM after closure had a distinct shape and scar tissue appearance compared to wounds treated with cBSA. The surface areas occupied by residual wound areas and scar tissues were significantly different between the cIM- and cBSA-treated wounds. Histological sections revealed larger scar tissue in the cIM-treated wounds, but the collagen fiber contents exhibited similar patterns in both cIM and cBSA-treated wounds. The results obtained by the authors are not sufficient for a complete conclusion on the use of colostrum fraction for wound treatment, and therefore, further research on the topic should be conducted.

The study by Çeleb et al. 2022 induced ulcerative mucositis in rats and divided them into two treatment groups, in addition to a control group. One group received bovine colostrum from the 1^{st} to 5^{th} day post-incision, while the other group received it from the 5^{th} to 10^{th} day. Histopathological and immunohistochemical analysis showed that both treatment groups exhibited ulcers, hemorrhagic areas, necrosis, and infiltration by mononuclear cells, as well as higher levels of TNF-α and 8-OHdG compared to the control group. There was no statistical difference in wound healing between the groups, but the treatment groups had a higher incidence of severe infections compared to the control group. The results did not indicate a significant reparative effect of bovine colostrum on mucositis and are limited by the study. Further studies are needed to elucidate the pathogenic inflammatory mechanisms of mucositis and the prophylactic and therapeutic roles of antioxidants.

However, other studies have obtained good results when using colostrum in wounds, such as the case of Kaur et al. (2022), who developed multifunctional nanofibrous dressings conjugated with bovine colostrum so that the bioactive factors of colostrum could act simultaneously at the wound site in a controlled manner. The developed dressing showed better cellular responses in terms of viability, spreading, and migration. In vitro studies confirmed the antioxidant and antibacterial properties of the colostrum-conjugated dressing. Moreover, rapid wound healing, better collagen formation, and anti-inflammatory responses in in vivo experiments confirmed better healing of rat wounds treated with colostrum-conjugated nanofibrous dressings. The bioactive factors present in colostrum aid the timely and orderly progression of the healing process. Therefore, bovine colostrum-based multifunctional wound dressings present a promising approach to overcome the challenges of traditional wound care (KAUR et al., 2022).

Tanideh et al. (2017), used a total of 80 adult female Sprague Dawley rats divided into four groups of 20 animals in each. without treatment (control), treatment with honey, treatment with colostrum and treatment with colostrum + honey. They created a circular wound in 2-cm diameter The progressive changes in wound were monitored by a camera on days 3, 7, 14, and 21 after operation. Complete dressing was performed with colostrum (0.5–1 ml) every day in one of the groups. The study took place for 21 days. at the end of the study, they observed that the topical application of colostrum helped in the wound reduction. however, the results were even better when they tested the synergistic effect of applying colostrum and honey together. This result opens the door to another interesting line of research, which is the evaluation of the effectiveness of the synergistic use of bovine colostrum with other bioactive compounds, with the aim of enhancing its beneficial effect on the recovery of damaged tissues.

Tarff et al. (2022) tested the action of colostrum as a topical treatment (in drops) in the healing of acute eye wounds after burns. Burns were created by applying 5 µl of 0.15 mol/L sodium hydroxide (NaOH) drops to the conjunctiva and corneal surface for 45 s in one eye of each animal, while the other eye was kept as a control, without burn and receiving only saline solution. After ten days of treating mice with 5 µl of colostrum in the experimental group, applied four times a day, the animals were euthanized, and eye tissues were removed from both the experimental and control groups.

Homogenized corneas (n=6 per group) were used to quantify pro-inflammatory and anti-inflammatory cytokines, namely tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), tumor growth factor-β (TGF-β), and interleukin-10 (IL-10), using Mouse ELISA MAX Deluxe Sets (BioLegend, San Diego, CA). Statistical

analysis was conducted using GraphPad Prism (Version 7.0, GraphPad Software Inc., San Diego, CA). Results showed that the application of BC significantly improved the fluorescence staining score for epithelial cells and conjunctival involvement in the corneas compared to those treated with SS after burns on days 4, 7, and 10. The BC treatment also suppressed tissue inflammation and neovascularization, and no significant difference was observed in corneal thickness between the burned and non-burned corneas.

Based on *in vivo* studies, it can be concluded that bovine colostrum is a promising natural source for wound healing. The bioactive factors present in colostrum help in the timely and orderly progression of the healing process. However, in some of the studies found in our search, colostrum did not bring any benefit related to tissue regeneration, although there is no explanation for this. More studies are needed to explore the full potential of colostrum in regenerative medicine and to develop more effective and targeted therapies for wound healing.

CONCLUSION

Studies related to the use of bovine colostrum in tissue regeneration are still scarce, and none of the studies found analyzed all stages, from colostrum collection to its proper use, including processing and composition. It is also possible to observe that concentrations that induce positive results do not show a dose-dependent effect, with very high concentrations inhibiting cell proliferation and differentiation, as well as very low concentrations. From the results found, it is evident that the application method also influences the final outcome, as specific portions showed positive results in contrast to the use of complete colostrum. This opens doors for experiments that can be performed with different vehicles, colostrum fractions, cell types, and concentrations, aiming to obtain better utilization of bovine colostrum properties in tissue regeneration.

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