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Mesenchymal Stem Cells as a Novel bio-regenerative Tool for Mastitis in dairy animals: A Review

Garima Kansal[a], Hariom Pandey[a], Vandana[a], Pradeep Chandra[b] and Brijesh Kumar[b]*

[a]Department of Livestock Production and Management, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India
[b]Animal Reproduction Division, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India

ABSTRACT

Mastitis, a complex multi-etiological disease has emerged as a major challenge and costly disease impacting the welfare of dairy animals across the globe. Different approaches from conventional antibiotics to most advanced biotechnological interventions have been tried to treat mastitis in dairy animals but none of these were able to regenerate the affected mammary gland. This non-regeneration of affected mammary glands leads to huge monetary losses to farmers in terms of loss of milk production, veterinary cost but also on the culling of lactating animals. The emergence of studies on mesenchymal stem cell and their potential regenerative and therapeutic role may prove a game changer in mastitis treatment in dairy animals. These cells have such inherent properties that make them a novel bio-regenerative tool for curing mastitis in dairy animals.

Keywords- *Antibiotics, Bio-regenerative tool, Mammary gland, Mastitis, Mesenchymal stem cells*

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CORRESPONDING AUTHOR:
Brijesh Kumar

E-MAIL ID:
brijeshivri@gmail.com

1. Introduction

Mastitis is a complex multi-etiological disease that has emerged as a major challenge impacting the welfare of dairy cattle across the globe. It accounts for about 40% of the total direct costs of the common production diseases [1]. Mastitis has public health significance due to its zoonotic potential as it is one of the derivatives for communicable diseases like tuberculosis, staphylococcal toxemia, septic sore throat, brucellosis, gastroenteritis, etc. [2]. Udder abnormalities caused by mastitis include marked swelling, increased warmth in acute and peracute stages, gangrene in some cases, and abscess formation while fibrosis and atrophy of glands in chronic cases [3]. Excessive formation of scar tissue in chronic mastitis, leads to morbidity and complete organ failure. For treating mastitis, the most prevalent managerial strategy is the intramammary infusion or intramuscular or intravenous injection of antibiotics, like streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline [4]. However, due to increasing antimicrobial resistance [5], and antibiotic residues in milk [6] leading to milk withdrawal and low cure rate [7], different approaches have been tried like vaccination, bacteriophages, natural plant, animal, and bacteria-derived products with their pros and cons. But none of the available therapeutic and curative approaches is able to improve or revert the post-mastitis structural damage of the mammary gland. Since the advent of stem cells, an increasing body of evidence indicates that the therapeutic use of stem cells helps in improving structural defects of tissue including mammary tissue affected with mastitis [8].

Stem cells are a type of blank cells having the ability to revive themselves through mitotic cell division i.e.self renewal and transformation into a wide variety of specialized cell types i.e. potency. The primary sources of stem cells are embryos, adult body organs, and fetus/cord cells. Adult stem cells which include mesenchymal, hematopoietic, and tissue-specific stem/progenitor cells [9] have been used for the treatment of animal diseases around the world. Mesenchymal stem cells (MSC) are multipotent cells with self-renewal and differentiation capability into mesodermal cell lineages [10]. MSCs are equipped with many important properties that make them precious in the field of cellular therapy and tissue engineering such as their low immune eliciting response, high anti-inflammation ability [11], ability to modulate innate immune responses [12], inhibit scar formation and apoptosis due to adhesion ability and mediating bioactive molecules, enhanced angiogenesis, and activation of intrinsic stem cells to regenerate their function [13]. Moreover, a wealth of information shows that MSC releases various antibacterial peptides (AP) including cathelicidin (CATHL2), indolamine 2,3-dioxygenase (IDO), and hepcidin (HEP) which helps in enhancing the clearance of bacteria [14]. It is also found that MSC derived from the bone marrow of bovine fetuses has an antiproliferative effect against Staphylococcus aureus cultured under in vitro conditions [15]. It has also been showing that BM-MSC has a unique property of transdifferentiating into the cells of ectodermal and endodermal lineages [16].

These stem cells can therefore be an important tool for

regenerative therapy of unresponsive debilitating diseases. After seeing its application and the need for alternative and/ or adjunct therapies for mastitis it can be presumed that MSCs may limit the destruction and restore the secretary tissue of the mastitic mammary gland and increase the chances of getting milk production potential to a near-normal level.

2. Mastitis and its type

Mastitis, the inflammation of mammary gland parenchyma is a complex multi-etiological disease described by various changes i.e. chemical, physical, and bacterial in milk and detrimental changes in mammary gland tissues affecting milk production and its quality. It is a global animal health problem affecting detrimentally the animal health, milk quality, and economics of milk production and every country suffers huge financial losses due to culling, less production, decreased fecundity, and treatment costs [17]. As per the present situation in India mastitis has represented itself as the most terrifying disease condition especially in high milk-producing animals just after Foot and Mouth Disease [18].

According to the clinical symptoms, mastitis may be classified as Clinical mastitis or Sub- Clinical mastitis. Clinical mastitis is characterized by sudden onset, inflammation, redness, edema of the udder, and reduced and altered milk secretions with flakes, clots, and off-color, bloody, or watery consistency from the affected quarters. In contrast, subclinical mastitis has no conspicuous signs both in milk and udder, but there is a decrease in milk quality and production. Subclinical mastitis can be detected by monitoring the number of somatic cells in the milk (Somatic Cell Count-SCC) [19]. According to Sheareand Harris, subclinical mastitis is 15 to 40 times more prevalent than the clinical form. Due to difficulty in diagnosing and persistence for longer periods subclinical mastitis leads to clinical form [20].

3. The economic concern of mastitis

Mastitis because of causing significant wastage and unacceptable milk quality, has emerged as a major challenge and continues to be one of the most widespread and costly diseases in dairy cattle across the globe accounting for about 40% of the total direct costs of the common production diseases [1]. After evaluating the monetary losses due to mastitis, it has been found to be due to reduced milk yield (up to 70%), premature culling (14%) milk discard after treatment (9%), and cost of veterinary services (7%) [21]. Worldwide, losses due to mastitis are estimated to be about US$ 35 billion and INR. 6000 crores for the Indian Dairy Industry annually [22]. Further, mastitis has public health importance due to its zoonotic potential as it is one of the derivatives for communicable diseases like tuberculosis, staphylococcal toxemia, septic sore throat, brucellosis, gastroenteritis etc. [2].

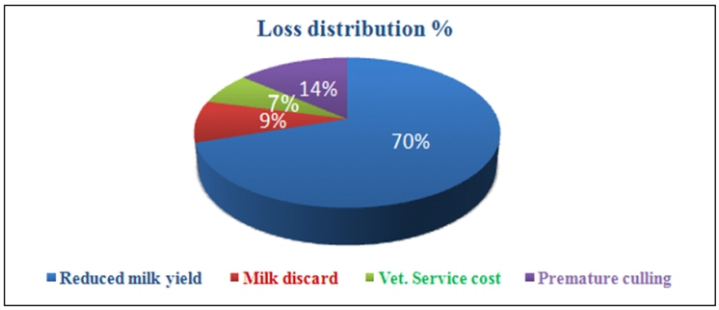

Fig1: Pie-chart showing economic loss distribution (%) due to mastitis

4. Causes of mastitis

Mastitis is caused by a wide spectrum of pathogens with considerably different aspects of infection like clinical vs. subclinical, acute vs. chronic with no classic exemplar having all possible aspects of the disease. Most pathogens associated with mastitis are of bacterial origin that can be categorized as contagious pathogens involving Streptococcus agalactiae, Staphylococcus aureus, and Mycoplasma bovis which reside mainly in the mammary gland and spread during milking and environmental pathogens involving Streptococcus species (Streptococcus uberis, and Streptococcus dysgalactiae) and environmental Coliforms (Gram-negative bacteria Escherichia coli, Klebsiella spp., Citrobacter spp., Enterobacterspp., Enterobacter faecalis and Enterobacter faecium and other gram-negative bacteria such as Serratia, Pseudomonas, and Proteus) [23]. Because of the presence of innate and specific immune responses, the bovine mammary gland is protected [24] but various external and internal factors could prevent this defense mechanism to perform its function properly [25].

One of the most usually isolated etiological agents causing one-third of clinical and subclinical mammary gland infections is S.aureus[26]. S.aureus mastitis is of special interest as they are the most difficult to treat because of its ability to internalize and persist within certain types of host cells so that the infection becomes subclinical. Also, the antibiotics do not always penetrate the host cells efficiently and even if they do, their concentration might not reach the level to destroy the pathogen [27] which eventually results in failure of antimicrobial therapy so that subclinical infections tend to become chronic.

5. Pathogenesis of S.aureus-induced mastitis

For establishing intramammary infections, the adhesion of bacteria is necessary for colonizing the mammary gland which is facilitated by the high surface hydrophobicity of S.aureus by making hydrophobic interaction with the cell membranes. Milk enhances the adherence [28] of S. aureus to mammary epithelial cells and disseminates to upper part of the gland by adhering to floating fat globules [29]. S.aureus produces several virulence factors including surface-associated secretory products, leukotoxins, enterotoxins (alpha and beta toxins) [30] and enzymes (hemolysins, leucocidin, coagulase, and nucleases) that help the pathogen to adhere to host membranes, resist phagocytosis, lyse eukaryotic cells and stimulates the release of series of immunomodulatory molecules in the host. Exotoxins produced by S.aureus damage the epithelial layer [31] and reveal the underlying subepithelial components e.g. fibrinogen and collagen which enables the adherence of surface proteins of staphylococcus to the host cell matrix [32].

S.aureus secretes various adhesion proteins like fibronectin-binding proteins (FnBP), fibrinogen binding protein (FbBP) and collagen binding protein which mediates the attachment to various cell surfaces proteins like collagen, fibrinogen and fibronectin [33]. The alpha toxin binds to the plasma membrane of epithelial cells and forms hollow hexameric complexes, which generate transmembrane pores, resulting in the leakage of low molecular weight molecules from the cytosol and ultimately cell death [34]. Beta toxin is a sphingomyelinase C in which hydrolysis sphingomyelins present in axoplasmic leaflet of the plasma membrane resulting in altered permeability with progressive loss of cell surface charge leading to rapid efflux of K+ and influx of Na+, Cl- and Ca+ [35]. The leakage of ions causes the breakdown of electric potential across the plasma

membrane decreasing negative charge on cell surface [36] and thus, influencing adhesion and invasion. Colonization of the mammary gland by S.aureus triggers the production of cytokines which elevates somatic cells primarily PMNs and this results in inflammation. This inflammation of the mammary gland does not eliminate bacteria totally which evolves to a chronic subclinical form that reflects a balance between the multiplication of bacteria and the mammary defenses. This balance can be because of less efficient phagocytosis due to the ingestion of casein and fat globules by PMNs[37] and by the production of antiphagocytic factors like protein A, superantigens, and capsule by S.aureus. Superantigens can stimulate macrophages and monocytes to produce various inflammatory mediators including tumor necrosis factor-alpha, nitric oxide, IL-6 and -10, and interferon-gamma in the presence of which macrophages become cytolytic. Also, superantigens facilitate S.aureus to evade the host defense system and contribute in both acute and chronic bovine mastitis.

6. Current Strategies to control mastitis

6.1. Antibiotics

Anderson tested the use of sodium cloxacillin against S.aureus-induced chronic mastitis in mice but the infection failed to respond to intramammary therapy with cloxacillin but responded against acute mastitis (38). Use of cefoperazone to treat mastitis in mice controlled the infection in the early stage but not during later stages [39], which was compatible with the treatment of delayed Staphylococcal mastitis cases in cattle [40]. Occasionally, bacteriostatic agents like macrolides and lincosamides which exert their action by inhibiting protein synthesis, were tested in mice. Pirlimycin showed more effectiveness against mastitis induced by β-lactamase producing strain of S.aureus which was due to more affinity for and prolonged retention in the mammary gland [41]. Routes other than intramammary like intravenous, intramuscular, and intraperitoneal have also been tried as they provide information about the capacity of compound to reach the mammary gland [42]. These alternative routes are effective and convenient as the intramammary route can be complicated because of the formation of a keratin plug in the mammary duct and the injections through this route increase the risk of contamination.
The antibiotic treatment may help in reducing the bacterial load but their extensive use also leads to drug resistance. It is not economical to treat S.aureus-induced mastitis with antibiotics because of the low bacterial cure rate [7]. Additionally, factors like pharmacokinetic problems, phagocytosis depressing effect of certain antibiotics [43], and their residues in milk curtails the use of antibiotics in mastitis. Hence, there is a need to find alternative strategies to control mastitis [44].

6.2. Vaccination

Because of the limitations of antibiotics, a shift has been made towards formulating vaccine to prevent the occurrence of mastitis. Various factors like vaccine type, cow's age and environment decides the impact of vaccines tested against S.aureus[45]. As example, killed bacteria-derived bacterins were found not fruitful in the prevention of new infections caused by S. aureus. Thus, DNA and recombinant protein vaccines were developed as alternatives to conventional therapy with bacterins from killed bacteria and antibiotics and these recombinant vaccines were found to be effective in protecting mammary glands against S. aureus infections [46]. E.coli J5 vaccine proved to be somewhat effective in decreasing the count and severity of coliform-causing mastitis by 70-80% [47]. Yet, for a number of reasons, vaccines developed for the prevention and control of mastitis have achieved only limited success. Certain factors that have curtailed the generation of effective vaccine against mastitis includes the complex etiopathology of mastitis, virulence factors, pathogenesis of causative agents and lack of understanding on immunobiology of mammary gland.

6.3. Bacteriophage therapy

Bacteriophage therapy can be an alternative to antibiotics in the fight against intramammary infections. Bacteriophages are viruses able to infect and kill bacteria [48]. Against S.aureus infections Phage K has been utilized as a prophylactic agent due to its lytic action against bacteria. However, to be successful as a therapeutic agent in mastitis its activity is must when it comes in contact with milk inside the mammary gland [49]. This proves to be a negative point for phage K that it gets inhibited by milk and other natural secretions from the udder [50]. Gill et al. also tested the efûcacy of phage K against S. aureus. This time also, phage therapy presented several limitations such as the degradation/inactivation of phage by milk and its components (whey proteins) and by the immune system [51].

6.4. Natural Compounds

Plants, animals and bacteria-derived biologically active agents can be a promising source as an alternate and/or adjunct to antibiotic therapy for mastitis. Fonseca et al. tested plant-derived diterpenes: manual, ent-kaurenoic acid and entcopalic acid against various bovine mastitis pathogens. Out of three, entropic acid (CA) was found the most active against most of the microorganisms tested [52]. Baskaran et al. also tested the antimicrobial activity of plant-derived molecules on a wide range of bacterial mastitis pathogens in milk, like streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, S. aureus, and E. coli. It was demonstrated through this in-vitro study that all plant-derived molecules (trans-cinnamaldehyde (TC), eugenol, carvacrol, and thymol) were effective against all microorganisms tested being TC found the most effective [53].
The immunomodulators (naturally produced by mammals) like lactoferrin, was used as potential antimicrobial agents for the treatment and prevention of bovine mastitis. Lactoferrin, a glycoprotein found in several body secretions exhibited an antibacterial effect against some major mastitis-causing pathogens like E. coli, S. aureus, coagulase-negative staphylococci, Pseudomonas aeruginosa and K. pneumonia[54]. Hafez et al. also isolated lactoferrin from bovine milk whey and tested its antimicrobial potential against S. aureus, E. coli, S. agalactiae, and P. aeruginosa[55]. Weisellaconfusa (lactic acid bacteria) and its metabolites found to be active against S. aureus and S. agalactia[56]. Bouchard et al. tested live lactobacillus casei as mammary probiotics which were able to prevent the internalization of S. aureus into mammary epithelial cells [57].
Unfortunately, none of the above-mentioned strategies is able to improve or revert the post-mastitis structural damage of the mammary gland. Since the advent of stem cells, an increasing body of evidence indicates that the therapeutic use of stem cells helps in improving structural defects of tissue including mammary tissue affected with mastitis [8].

7. Stem cells: A potent bio-regenerative tool

Stem cells have been a focus of intense research and publicity for

the last decade. Stem cells are considered today as the most assuring cells because they can divide without limit to replenish another type of cells in the body as a backup for the repair system. These undifferentiated cells found in the embryonic, fetal, and adult stages of life give rise to differentiated cells that are building blocks of tissue and organs. The three major characteristics of stem cells are: (a) self-renewal (proliferation), (b) clonality (usually arising from a single cell), and (c) potency (differentiation ability) [58]. Stem cells can be of four types depending on their origin: Embryonic stem cells, fetal and adult stem cells, and induced pluripotent stem cells [59]. MSCs are relatively easy to isolate and expand in culture with no ethical issues as with ESCs. MSCs have been shown to differentiate into the cells of mesodermal, endodermal, and ectodermal lineages which were isolated from different species including bovines [60,61]. The most pertinent difference between MSCs and ESCs that make MSCs favorable for clinical use is their property of not forming teratomas when injected in vivo [62].

7.1. Characteristics of MSCs

The International Society for Cellular Therapy (ISCT) set up three basic parameters to define minimal characteristics of MSCs [63] : under standard culture conditions, MSCs should display plastic adherence, and must express cell peculiar markers like CD105, CD73, and CD90, but lacks the expression of other hematopoietic cell surface markers such as CD45, CD34, CD14 or CD11b, CD79 or CD19, HLA-DR, should be capable of differentiation into osteoblasts, adipocytes, and chondroblasts in vitro under a specific set of culture conditions. Morphologically they are characterized by a small cell body with a few long and thin cell processes. A large round nucleus with a prominent nucleolus surrounded by chromatin particles is present in the cell body. The cell body also contains a small amount of Golgi apparatus, rough endoplasmic reticulum, mitochondria, and polyribosomes. There is a wide dispersion of these cells with few reticular fibrils present in the extracellular matrix but lacks other types of collagen fibrils [64]. MSCs have been shown to differentiate into the cells of mesodermal, endodermal, and ectodermal lineages which were isolated from different species including bovines[60,61].

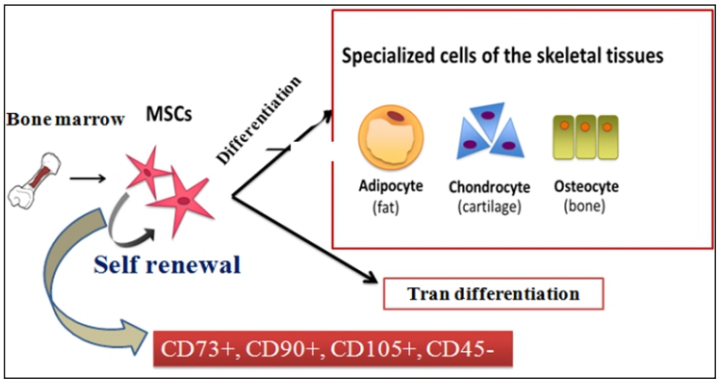

Figure 2: Characteristics of mesenchymal stem cells

7.1.1. Paracrine Signaling of MSCs

MSCs exhibit their healing effects not through differentiation potential or engraftment but rather due to paracrine signaling and communication through cell-cell contact [65]. MSCs treatment for myocardial infarction, graft versus host disease (GvHD), and autoimmunity disorders (Crohn's disease and type I diabetes) are some of the instances of paracrine-mediated treatment of MSCs [66]. The secretome of MSCs contains a wide range of molecules that are useful for tissue repair, including ligands that stimulate the proliferation and differentiation of other stem cells, chemoattraction, antifibrosis, antiapoptosis, angiogenesis, and immunomodulation [67].

7.1.2. Immunomodulation

The most impactful property of MSCs is their immunomodulatory capacity which is partly attributed because of their inhibitory effect on T-cell activation and proliferation, both directly through cytokines and indirectly through modulating regulatory T-cell activity [68]. MSCs also modulate the behavior of natural killer cells, B-cells, dendritic cells, neutrophils, monocytes/macrophages through the actions of a number of molecules like prostaglandin E2 (PGE2), nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO), interleukin-10 (IL-10), and many more [67]. MSCs have also been involved in stimulating alternative activation of macrophages towards the M2 phenotype which is regenerative as well as proangiogenic M2, as against the classical proinflammatory M1 phenotype [69] which helps in localized tissue repair. Hence, adult MSCs in vivo also play role in coordinating healing responses and preventing autoimmunity after injury, immune-mediated diseases and transplantation rejections [65]. MSCs are not solely anti-inflammatory but can elicit inflammatory responses under certain conditions by presenting antigens to induce CD8+ T-cell responses, increasing expression of MHCDII, and presenting antigens to CD4+ T-cells [70].

7.1.3. Anti-apoptotic activity

MSCs check cell death via rehabilitation of the local microenvironment by producing proteins that are inhibitors of apoptosis and by increasing the expression of anti-apoptotic proteins [71]. Hence, it was reported that the pro-apoptotic factors Bax and cleaved caspase-3 expression were decreased but the anti-apoptotic Bcl-2 level was increased by MSC. The expression of pro-angiogenic factors, such as basic fibroblastic growth factor (bFGF), vascular endothelial growth factor (VEGF), and CXCL12 were increased in MSC-treated hearts compared to medium-treated hearts[72].

7.1.4. Tissue regeneration

MSCs secrete trophic factors such as stromal-derived factor-1 (SDF-1), insulin-like growth factor (IGF-1), epithelial growth factor (EGF), hepatocyte growth factor (HGF), nerve growth factor (NGF), transforming growth factor-alpha (TGFa), vascular endothelial growth factor (VEGF) which helps in enhancing cell survival [73]. After inducing bronchopulmonary dysplasia in a rodent it was found that SDF-1 knocked down MSCs showed significantly less beneficial effects in repair processes like alveolarization, angiogenesis, and inflammation marked by infiltration of macrophages in alveolar spaces in comparison to non-silenced control MSCs [74]. MSCs also possess the ability to migrate toward the sites of injury through chemo-attractant gradients present in the extracellular matrix and peripheral blood vessels. Local factors in these injury sites like hypoxia, cytokines, and toll-like receptor ligands can stimulate MSCs functions. All these stimuli promote the formation of growth factors by MSCs that augment tissue regeneration [73]. MSCs secretions also have anti-fibrotic and angiogenic effects that can reduce scar formation [75]. Lange-Consiglio et al. investigated the effect of conditioned media

derived from amnion in an in vitro experiment on mammary epithelial cells infected by S. aureus and in an in vivo study on cows with acute and chronic mastitis. Their results showed that conditioned media is able to attenuate the proliferation of S. aureus in bovine mammary epithelial cells (BME-VU)cells either when added at time 0 or when supplemented 4h after the cells were exposed to this bacteria. In vivo, study results showed somatic cell count (SCC) values decreased markedly at 30 days of observation (T 30) in both mastitis groups, and no statistically significant differences were observed between antibiotic and conditioned media-treated quarters. However, the conditioned media group had a lower rate of relapses. Indeed, mastitis did not recur in any of the quarters after conditioned media treatment [76].

7.1.5. Angiogenesis regulation

The activation of angiogenesis is pivotal for the nutrition of damaged tissue and hence to the recovery of ischemic areas. Hypoxic and inflammatory conditions induce the expression of VEGF and angiopoietins [77] which activate endothelial progenitor cells that will constitute new vessels. The divergent population of MSCs like adipose, bone marrow, Wharton jelly, and umbilical vein lead to the proliferation and migration of endothelial cells promoting tube formation, as well as preventing endothelial cell apoptosis in vitro [78]. It has been demonstrated that the application of MSCs has been proven successful to promote angiogenesis in different animal models of cerebral ischemia/stroke, myocardial infarction, neurogenic bladder, and urinary incontinence [79]. Tissue inhibitor of metalloproteinase-1(TIMP-1) is recognized as the molecule accountable for the anti-angiogenic effects of MSCs when the proteomic analysis was done by inflammatory cytokine-stimulated MSC-CM [80].

7.1.6. Antimicrobial activity

Many studies indicate that MSC promotes the removal of bacteria because of the release of various antibacterial peptides (AP) like cathelicidin (CATHL2), indolamine 2,3-dioxygenase (IDO), and hepcidin (HEP) [81,82,14]. A membrane pore-forming AP CATHL2, LL-37 from human MSCs has been shown to restrict the growth of Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus under in vitro conditions [81]. IDO, a key mediator of immune tolerance can deplete tryptophan in MSC culture because of its activity as a catabolic enzyme and hence hinders the growth of S. aureus, Staphylococcus epidermidis, Enterococcus faecium, and E. coli [82]. HEP is an iron regulator hormone hence restricting the availability of iron for the growth of microorganisms [83]. It has been reported that MSC when encountering bacteria bring some changes in their secretions and their activities like migration, expansion, and distinctness [84]. The encounter of human MSC with S.aureus increases the release of several paracrine factors including VEGF, SDF-1, and IL-6, which leads to the influx and activation of inflammatory cells to infected tissue [85]. In vivo experiments indicate that MSCs treatment helps in the reduction of infections caused by bacteria and inflammatory response in rats when they were infected with S. aureus which is resistant to methicillin [86]. CATHL2 released by human and equine MSC has been intricated in the antibacterial capacity against S. aureus[87-88]. CATHL2 is also released by bovine mammary epithelial cells and is stimulated in the presence of S. aureus [89].

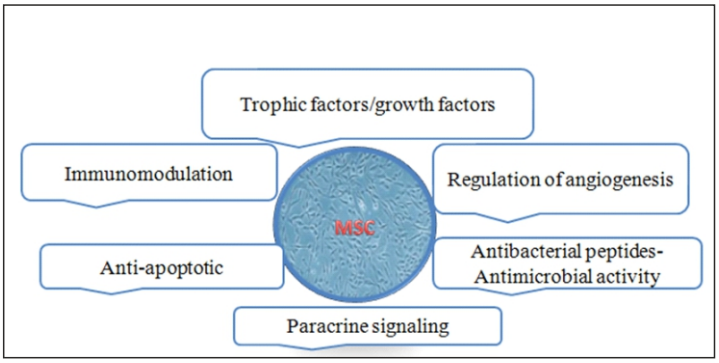

Figure 3: Different properties of mesenchymal stem cells

7. Stem cells as therapy in mastitis

Mastitis control is mainly dependent on the use of chemical disinfection, antiseptic teat dipping, and antibiotic therapy, but persistent use of antibiotics can result in antibiotic resistance in microbes [5], eventually leading to conventional antibiotic therapy failure. Further, the pathogenic bacteria and antibiotic residues present in mastitic milk made it unfit for consumption and spread several diseases like tuberculosis, brucellosis, scarlet fever, gastroenteritis, food poisoning, staphylococcal toxemia, etc [90]. Hence, many alternative therapeutic agents have been tried like vaccination, bacteriophages, and naturally derived products of plants, animals, and bacteria which all prove advantageous at some level. But none of the therapeutic and preventive approaches is able to bring back the post-mastitis degenerated tissue to its near-normal architecture and production. Since the dawn of stem cells, a growing body of evidence proves that the use of stem cells in therapy improves structural defects of tissue. Stem cells are being explored for a number of chronic degenerative diseases that have so far escaped remedial measures from traditional antibiotic approaches with the hope that cell therapy would repair, repopulate and replace tissues and organs regenerating hope. There are few studies that reflect the therapeutic effects of stem cells in mastitis treatment in dairy animals.

Table1: Different studies reflecting the application of stem cells in mastitis

Study by	Criteria of selection	Therapy	Results
Costa et al. [91]	8 lactating goats were selected after the diagnosis of mastitis.	The cell therapy (3.5 x 106) was performed in five areas directly at the left mammary gland	Differences were found in the echo of healthy and affected mammary tissue, and physicochemical parameters of milk among the groups (Control, chronic mastitis group with no stem cell treatment, animals with chronic mastitis treated with ASCs). In histopathological evaluation there were alterations in the mammary parenchyma of the animals affected by mastitis before and after cell therapy, reduction in infiltration of inflammatory cells and fibrosis was observed after the application of ASC

Peralta et al. [92]	15 Holstein Friesian cows at first lactation. Cows were inoculated in the two left quarters with a suspension of S. aureus and were randomly assigned to three experimental groups of 5 animals each.	Cows were inoculated with S. aureus and treated intramammary with vehicle (NEG; days 4 and 10), antibiotics (ATB; days 4 and 5), or a suspension of 2.5 × 107 AT-MSCs (MSC; days 4 and 5).	Intramammary administration bovine fetal AT-MSCs in mastitis cows did not induce changes in clinical variables or haptoglobin and amyloid A serum concentrations but resulted in lower bacterial count in milk compared to cows treated with vehicle
Ting et al. [93]	The Holstein cows with mastitis were divided into two groups 1) antibiotic control group (tetraneomycin) ointment 2) conditioned-Dulbecco's pluripotent stem cells (DPBS) from the amniotic membrane (AMSCs) treated group	A control group was treated with antibiotics while the experimental group was treated with conditioned-DPBS, obtained from AMSCs culture. Antibiotic ointment and conditioned DPBS from AMSCs were injected into the mammary gland through the nipple and milk duct.	Mastitis treatment with conditioned-DPBS from AMSCs (experimental group) and conventional antibiotics (control group) showed insignificant differences in pH value and titratable acidity. The level of ionic calcium concentration in the conditioned-DPBS group decreased from the 3rd day to the 12th day, while the level in the antibiotic group decreased from 0 day to the 12th day. The somatic cell number was similar in both groups, which meets the standard of Taiwan milk collection

7. Future scope

After several decades of experiments, stem cell therapy is becoming a magnificent game changer for medicine. With each experiment, the capabilities of stem cells are growing, although there are still many obstacles to overcome. Regardless, the influence of stem cells in regenerative medicine and transplantology is immense. Stem cell research offers a wide scope, such as for the treatment of infertility, knowing the causes of congenital disease, acting as a candidate in therapeutic drugs, in developmental biology, a vehicle for delivering genes, developing treatments for serious diseases, helps to develop improved methods of cloning by nuclear transfer in farm animals to produce high-quality genetic strains -free from diseases, to aid research and development of new drugs, to characterize gene expression profile. Understanding different molecular processes related to the functions of stem cells will allow us to harness their potential and develop strategies that control their differentiation. In spite of being one of the important upcoming areas in biological research, enthusiasm towards the better application of this technology in regenerative medicine is yet to be expanded like many other new and exciting technologies, soon stem cell technology is likely to become a natural extension of therapeutics in the veterinary application. Despite recent advances in veterinary regenerative medicine, the discipline is still in its early stages, and much more research is needed to answer many concerns before being proven. New regenerative medicines are on the increase, and we live in exciting times. One might expect that ongoing research in this area will lead to a moment where stem cell treatments for many now incurable diseases will no longer be a pipe dream but a viable and accessible option for patients in both veterinary and human medicine.

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