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Original Research Article

Phytochemical Screening and Antibacterial activity of Okra extract

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Abstract: Okra (*Abelmoschus esculentus*) belongs to malvaceae family and its widely cultured in the world for its fibrous fruits which contain round white seeds. In this study the bioactive compounds where extracted by using many solvents methanol, ethanol, ethyl acetate and water. The ethanolic extract where used invitro to determine the antibacterial activity of the extracts of dried fruit okra against some selected potential bacterial pathogens, *Klebsiella* and *E. coli*. This research is an experimental study using completely randomized design by using disk diffusion testing method. The object used was the extract of the Okra fruits at concentration of 25%, 50%, 100% and 200%. The zone of inhibition was 3.73mm at 200% with *Klebsiella* and 3.47 mm with E. coli in comparison with the control 4.4 mm. The least concentration of the extract that completely inhibit the growth of the organism MIC is 30%.

Keywords: Okra, klebsiella, E. coli antibacterial activity.

INTRODUCTION

The reduced susceptibility of the bacteria to the antibiotic become a problem worldwide. Also, the increased toxicity of scientific drugs has led to using natural, safe potent antibacterial agents rather than scientific drugs (Gottlieb *et al*., 2002 and Narod *et al*., 2004). Scientists direct to dissolve the problem by use fungi, algae and higher plants to develop new antibiotics. Large number of organic bioactive compounds are produced by these higher plants as secondary metabolites, which used in synthesis of chemotherapeutic, bactericidal, and bacteriostatic agents (Evans *et al*., 1986 and Purohit *et al.*, 1998). In recent years the researchers attention directed toward the identification of antibiotics from plants because, plant derived antibiotics still remain an area of intensive investigation (Cutter, 2000, Jain, *et al*., 2010 and Shirazi *et al*., 2007). In several medicinal applications Okra mucilage has been used (Kumar, 2010). Abelmoschus esculentus is a vegetable crop where the immature pods used in syntehsis of soap and stew. It is called as ladyfingers, gumbo and bhindi. The trop ical and subtropical parts of the world are the main area of its growth such as Nigeria, India, Ethiopia, Turkey, Japan, Malaysia and the south united states (Khomsug, *et al.,* 2010 and Nwangburuka *et al*., 2013). It is rich in vitamins, minerals (iron, potassium, manganese and calcium) and dietary fat. It has been used in genitourinary disorders, in controlling cholesterol and hypertension level, chronic dysentery, ulcer and anti- inflammatory properties (Ansari *et al*., 2005). This study has been conducted to assess the inhibitory activity of ethanolic extract of A. esculentus pods against selected pathogenic bacteria *E. coli* and *Kleibsiella* species.

MATERIAL AND METHODS

Preparation of Extract

150 grams of each selected dried plant powder were weighed and added to a 600 ml of ethanol, in a conical flask of a 1000 ml capacity. The flask was covered and left a side for a 24 hour. The plant mixture was mixed using magnetic mixer and filtered then the plant extract was kept in the refrigerator until use.

Preliminary Phytochemical Screening

Screening of the above selected medicinal plant for various phytochemical constituents were carried out using standard methods (Dibyajyoti *et al*., 2011). Qualitative phytochemical screening of plant extracts was carried out using the following methods to test only the presence of secondary metabolites by using different solvents.

Test for Tannins

0.008 M Potassium ferricyanide was added to 1 ml of the extract in a test tube, 1 ml of 0.02 M Ferric chloride containing 0.1N hydrochloric acid was also added. A blue-black coloration was observed.

Test for Flavonoids

Crude extract was added to 5 ml of diluted ammonia solution and concentrated H2SO4. The presence of flavonoids is indicated yellow coloration which disappeared on standing.

Test for Alkaloids

In 2ml of 1% HCl crude extract was dissolved and gently heated.To the mixture Mayers reagents were added to the mixture. The presence of alkaloids confirmed by the turbidity of the resulting precipitate.

Thin Layer Chromatography (TLC) Analysis

The Ethanolic, Methanolic, Ethyl acetate and Water Okra extracts were loaded on silica plate (Merck Aluminium sheet—silica gel 60 F 254). A mixture of H:C:M (1:1:1), P: E: W (1:2:1), M: E: W (1:1:2) and P:M: W (3:1:1) were used as the solvent system. The TLC plate was kept in iodine chamber for one minute and under UV light (254 nm) to visualize bands on chromatogram (Asha *et al.*, 2013 and Das *et al*., 2010).

Preparation of Bacterial Isolates

Two different types of bacterial strains were obtained from the medical laboratories which are Escherichia coli and Klebsiella.

Screening of antimicrobial activity

Media for test organisms

36 g of Muller Hinton Agar was added to 1000 ml of sterile distilled water and autoclaved at 121^{o} C for 30 minutes at 1.5 lbs. After cooling both the agar was poured into sterile Petri plates approximately 4mm and allowed to set at ambient temperature and used. Sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had at most 4 holes of 7 mm diameter and 5 mm depth made into it using a sterile agar glass borer. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002).

Inhibition Activity of Different Concentration of Okra Extracts

This was carried out using agar well diffusion method. 200 µl of different concentration of the aqueous and ethanoic extracts (25mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of Okra pods were dispensed separately in wells already seeded with the test isolates and incubated at 37°C for 24 h. After incubation, the inhibitory activity of the minimum concentration of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter. Standard antibiotic discs were used as a positive control to compare the antibacterial activity. The discs loaded with test extracts, and the standard antibiotic were placed with help of sterile forceps carefully with adequate spacing between each other. After incubation, the antibacterial activity of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the crude leave extract of Okra was determined by using the method of Greenwood (1989) as described by (Geidamet *et al*., 2007). Serial dilution of the extract at the concentrations of 25, 30,35, 40, 45,50,100 and 200 mg/ml. Where 18 mg of the Muller Hinton Agar media was prepared in 500 ml of distilled water and autoclaved at 121º C and 5Ib for 30 minutes then cooled, the media filled in tubes each tube contain 17 ml. Astandarazed inoculum for each bacterial strain was prepared to give an inoculum size approximately 10^{-5} in 5 tubes each tube contain 10 ml of distilled water. Put each extract concentration in the tube containing 3ml of distilled water and mixed properly then taken off by a sterile syringe and filtered by filter paper and add the prepared M.H.A broth and mixed properly then add 100 micro of bacterial isolate and mixed again then put them in autoclaved petri dishes and move the dishes in different directions to homogenize the plant extract. The control sample containing only the bacteria without extract. Then kept at 37 C for 24 hrs. in incubator. Then determine minimum inhibitory concentration and recorded as the least concentration of the extract that completely inhibit the growth of the organism.

Determination of Minimum Bactericidal Concentration (MBC):

Two nutrient agar plates were prepared. The bacterial isolate of kliebsiella incubated for 24 hr. then 10^{-5} serial dilution were prepared. The diluted bacterial isolate spread on one plate in different direction. The other plate cultured by non-diluted bacterial isolate. 200mg/ml of ethanol extract mixed with 3ml of distilled water. 5 mm size discs from filter paper were cut and filled with the extract and then put them on the agar as well as antibiotic discs and then kept in the incubator for 24 hr at 37º C with a control plate. The lowest concentration with no visible growth was defined as MBC, indicating 99.5 % killing of the original inoculum.

RESULT AND DISCUSSION

Phytochemical Screening of Sequential Extracts of Okra

The results of plant extract under investigation are shown Table 1. leaves extract showed positive result for the presence of medicinally active constituents. In the Water extract; tannins, phenolic compounds, flavonoids, alkaloids, were the most common present in the tested plants. While phenolic compounds are absent in methanolic, ethanoic and Ethyl acetate extract. Plants which rich in a wide variety of secondary metabolites, such as terpenoids, alkaloids, tannins, flavonoids appear biological and pharmacological activities and may have potential to be used as chemotherapeutic agents or serve as starting material in the developing of new antibiotics.

Table 1: Preliminary phytochemical screening of Okra extract

leaves methanolic extract	phytochemical compounds of Okra			
	Phenolic compound	Flavonoids	Alkaloids	Tannins
Methanolic extract	**-ve**	**+**	**-ve**	**+**
Ethanolic extract	**-ve**	**-ve**	**+++**	**+++**
Ethyl acetate extract	**-ve**	**+**		**-ve**
Water extract	**+++**	**+**	**+++**	**+++**

(+++) high (++) medium (+) poor (-) no found

Thin layer chromatography profiling several bands or spots were observed during partitioning of extract components with mobile phases systems indicating separation of bioactive compounds depending on polarity (10). The RF values are shown in table 2 of methanolic, ethanolic, ethyl acetate and water extracts.

Table 2: Thin Layer Chromatography

	R_f values			
	Methanolic Extract	**Ethanolic**	**Ethyl acetate**	**water extracts**
H:C:M(1:1:1)	**0.25, 0.29**	**0.085, 0.148**	**0.106, 0.212, 0.97**	**0.063, 0.074, 0.106**
P:E:W(1:2:1)	**0.71, 0.77, 0.82, 0.91**	**-**	**0.68, 0.77**	**0.77**
M:E:W(1:1:2)	**0.028, 0.074, 0.048**	**0.029, 0.0102, 0.42**	**0.075, 0.45, 0.54**	**-**
P:M:W(3:1:1)	**0.45, 0.72**	**0.5, 0.67, 0.85**	**0.45, 0.82**	**-**

Antibacterial activity of the pods extract

The present study was on the determining antibacterial activity using agar well diffusion method by measuring the inhibition zone in mm against two bacterial strain E. coli and Kleibsiella species and phytochemical screening in Leaves of Okra with different solvents water, 70% ethanol, 80% methanol and petroleum ether. The extract used in this study was the ethanolic extract. The potency of the ethanolic extract A. esculentus pods against Ecoli and Klebsiella was examined based on the presence and absence of of zone of inhibition measured in diameters as shown in table 3. In the search, plant parts play important role because their huge production of organic compounds for medicinal use. The ethanolic extract of the Okra pods exerted inhibitory properties against the test bacterial isolates (E. coli and Klebsiella). This could be due to presence of bioactive compounds in most plant parts which show antibacterial activity (Pereira JA *et al.*, 2007). Results of research on the growth of E. coli and Klebsiella, by using the disk diffusion disk and measuring the inhibitory zone, have revealed that the Okra extract can inhibit the growth of the two microorganisms. The optimum concentration to inhibit the growth of E. coli at 100 mg/ml and 200 mg/ml with zone 2.74 mm and 3mm respectively, where for the Klebsiella the zone was 2.37 mm and 3 mm respectively in comparison with the control inhibitory zone was 4.4 mm. However, there is no inhibition at concentration of 25 and 50 mg/ml. This indicate that Okra fruit has optimum concentration to suppress the growth of E. coli and Klebsiella bacteria which can be seen from the inhibition zone diameter Fig-1 and Fig-2 for E. coli and Klebsiella respectively.

Fig-1: Zone of inhibition for E. coli, positive control of chloramphenicol (Middle), (1) for 25 mg/ml, (2) for 50 mg/ml (3) for 100 mg/ml and (4) for concentration of 200 mg/ml

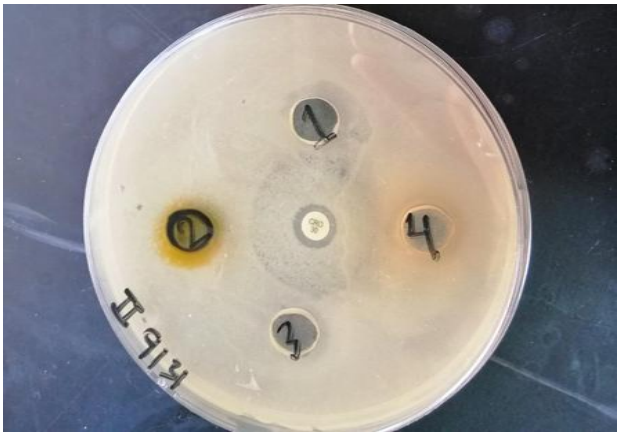

Fig-2: Zone of inhibition for Klebsiella, positive control of chloramphenicol (Middle), (1) for 25 mg/ml, (2) for 50 mg/ml (3) for 100 mg/ml and (4) for concentration of 200 mg/ml

In addition to the factor of concentration, the ability to inhibit bacterial growth also determined by antimicrobial material substance which produced by the plant (Rastina *et al*., 2015). In this research, the antibacterial was due to the presence of bioactive compounds such as flavonoids, tannins. Saponins, in okra fruit (Septianingrum *et al*., 2018). Due to the interaction between flavonoids and bacterial DNA the flavonoids cause damage to bacterial cell wall, microsomes and lysosomes (Nagappan *et al.*, 2011). In addition, flavonoids have lipophilic characteristics therefor they have ability to damage the cell membrane of bacteria (Rianto *et al*., 2015). Moreover, flavonoids are also important as a powerful antioxidant in decreasing the risk of chronic diseases, the cancer process, anti-inflammatory, antibacterial, and antiallergic. The antibacterial action of flavonoid substances thought to degradation of bacterial cell proteins and damage cell membrane beyond repair (Sudoyo, 2009). In this study tests one way Anova showed the calculated p value for the Ecoli bacteria was 0.000 at the concentration 200%. Whereas the p value for the Klebsiella was 0.001 at same concentration so that the okra pud extraction can inhibit the growth of both gram negative bacteria (E. coli and Klebsiella). Positive control showed more antibacterial activity against test bacteria compared with tested samples.

Table 3: Antibacterial Activity of Okra pods Extract against bacteria (E.coli and Klebsiela) concentration (µg /mL)/ Zone of inhibition (mm)

Organism	**25**	**50**	**100**	**200**	**Control**
E.coli	no	no	2.47 ± 0.06	3.00 ± 0.00	4.40 ± 0.00
P-value	no	no	0.000	0.000	
Organism	25	50	100	200	Control
Klebsiella	no	no	2.37 ± 0.16	3.00 ± 0.00	4.40 ± 0.00
P-value	no	no	0.000	0.001	

The minimum inhibitory concentration (MIC) value of ethanolic extract of Okra pods against Kleibsilla. According to Table the MIC value of ethanolic extract treated on Kleisilla was found to be 30 mg /ml.

MIC of ethanoic okra leaf extract:

Extract concentration	**MIC**
45	no
40	no
35	10
30	no
25	20
20	23
15	30
5	90

The Minimum Bactericidal Concentration (MBC) Klebsiella was 30 mg/ml. This was the lowest concentration, from which there was no bacterial growth during MIC determination. The plates were examined after 24 hours incubation of the test organisms. The result revealed that MBC equals to MIC.

MBC of ethanolic okra leaf extract:

Concentration of extract	**No of colonies**
45	no
40	no
35	10
30	no
25	20
20	23
15	30
5	90

CONCLUSION

The phytochemical compounds present in the okra pods extract exhibits antibacterial activity. That could prove the plant extract as potential natural antibacterial agent. More research work can be carried out on the isolation and characterization of bioactive compounds present in A. esculentus pods for better therapeutic use against pathogenic bacteria.

REFRENCES

- Ansari, N. M., Houlihan, L., Hussain, B., & Pieroni, A. (2005). Antioxidant activity of five vegetables traditionally consumed by south- Asian migrants in Bradford, Yorkshire, UK. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(10), 907-911.
- Asha, V., Sudhanshudhar, D., & Namrata, S. (2013). Spectral analysis of steroidal saponin isolated and purified from leaves extractf Asparagus racemosus (Family—Asparagaceae), *American Journal of Advanced Drug Delivery*, 1(5), 770–776.
- Cutter, C. N. (2000). Antimicrobial effect of herb extracts against E. coli 0157.H.7. Listeria monocytogensis and Salmonella typhimurium associated with beef. *J Food Protect*, 63, 601–607.
- Cheesbrough, M. (2002). Medical laboratory manual for tropical countries. ELBS edition. Tropical health technology publications, UK. 2:2-392.
- Dibyajyoti, S., Bindu, J., & Jain, K. V. (2011). Phytochemical evaluation andcharacterization of hypoglycemic activity of various extracts of Abelmoschusesculentus linn. fruit, *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(2), 183.

- Das Talukdar, M., Dutta, C., & Chakraborty, M., & Dutta, B. K. (2010). Phytochemical screening and TLC profiling of plant extracts of Cyathea gigantea (Wall. Ex. Hook.) Haltt and Cyathea brunoniana. Wall. ex. Hook. (Cl. & Bak.), Assam University, *Journal of Science & Technology*, 5(1), 70–74.
- Evans, J. S., Pattison, E., & Moris, P. (1986). Antimicrobial agents from plant cell culture, in secondary metabolites in plant cell culture (edited by Moris PA, Scraggs A, Stafford A, Flower M) Cambridge University, London.
- Gottlieb, O. R., Borin, M. R., & de Brito, N. R. (2002). Integration of ethnobotany and phytochemistry. *Dream or reality Phytochemistry*, 60, 145–152.
- Geidam, A. Y., Ambali, A. G., & Onyeyili, P. A. (2007). Phytochemical screening andantibacterial properties of organic solvent of fraction of Psidium guajava aqueous leaf extracts. *International Journal of Pharmacology*, 3, 68–73.
- Jain, P., Bansal, D., Bhasin, P. A., & Anjali, A. (2010). Antimicrobial activity and phytochemical screening of five wild plants against Escherichia coli, Bacillus subtilis and Staphylococcus aureus. *J Pharm Res*, *3*(6), 1260-1262.
- Kumar, S., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, N., & Kouame, C. (2010). Okra (Abelmoschus spp.) in West and Central Africa: potential and progress on its improvement. *African Journal of Agricultural Research*, 5(25), 3590-3598.
- Khomsug, P., & Thongjaroenbuangam, W. (2010). Antioxidative activities and Phenolic content of extracts from Okra (Abelmoschus esculentus L.). *Research Journal of Biological Sciences*, 5(4), 310–313.
- Narod, F. B., Gurib-Fakim, A., & Subratty, A. H. (2004). Biological investigations into Antidesma madagascariense Lam. (Euphorbiaceae), Faujasiopsis flexuosa (Lam.) C. Jeffery (Asteraceae), Toddalia asiatica (L.) Lam. and Vepris lanceolate (Lam.) G. Don (Rutaceae). *Journal of Cell and Molecular Biology*, 3, 15–21.
- Nagappan, T., Ramasamy, P., Wahid, M. E. A., Segaran, T. C., & Vairappan, C. S. (2011). Biological activity of carbazole alkaloids and essential oil of Murraya koenigii against antibiotic resistant microbes and Cancer cell lines. *Molecules*, 16(11). doi: 10.3390/molecules16119651
- Nwangburuka, C. C. (2013). Cytomorphological and antifungal analysis of Acalypha wilkesiana and Moringa oleifera extracts, and sodium hypochlorite on Abelmoschus esculentus L. Moench. treated seeds. *Nature Sci*, *11*, 31-39.
- Pereira, J. A., Oliveira, I., Sousa, A., Valentão, P., Andrade, P. B., Ferreira, I. C., ... & Estevinho, L. (2007). Walnut (Juglans regia L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food and chemical toxicology*, *45*(11), 2287-2295.
- Purohit, P., & Bohra, A. (1998). Effect of some plants extracts on conidial germination of some important phytopathogenic fungi. *Geobios New Report*, 17, 183-184.
- Rianto, L., Handayani-Indri, A., & Septiyani, A. (2015). Uji Aktivitas Ekstrak Etanol 96% Biji Srikaya (Annona squamosa l.) Sebagai Antidiare yang disebabkan Oleh Bakteri Shigella dysenteriae dengan Metode Difusi Cakram. *Jurnal Ilmiah Manuntung*, 1(2), 181-186.
- Rastina-Mirnawati, S., & Ietje, W. (2015). Aktivitas Antibakteri Ekstrak Etanol Daun Kari (Murraya koenigii) Terhadap Staphylococcus aureus, Escherichia coli, dan Pseudomonas sp. *Jurnal Kedokteran Hewan*, 9(2), 185-188
- Shirazi, M. H., Ranjbar, R., Eshraghi, S., Sadeghi, G, Jonaidi, N., Bazzaz, N., Izadi, M., & Sadeghifard, N. (2007). An Evaluation of Antibacterial Activity of Glycyrrhiza galbra Extract on The Growth of Salmonella, Shigella and ETEC E.coli, *Journal of Biological Sciences*, 7(5), 827-829.
- Septianingrum, N. M., Hapsari, W., & Syariffudin, A. (2018). Identifikasi Kandungan Fitokimia Ekstrak Okra Merah (Abelmoschus Esculentus) dan Uji Aktivitas Antibiotik Terhadap Bakteri Escherichia coli. *Jurnal Insan Farmasi Indonesia*, 1(2). doi:10.36387/jifi.v1i2.240
- Sudoyo A, W. (2009). Buku Ajar Ilmu Penyakit Dalam Jilid II. Interna Publishing: Jakarta.

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Research Article

Stem Cells: Insights into Niche, Classification, Identification, Characterization, Mechanisms of Regeneration by Using Stem Cells, and Applications in Joint Disease Remedy

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Abstract

Background: Stem cell therapy has attracted much interest in the 21st century, not only because of the controversy surrounding the ethics involving pluripotent stem cells, but their potential for clinical use.

Objectives: The present review highlights the stem cells niche, types, identification, and characterization, mechanisms of regeneration by using stem cells, and applications in joint disease remedy. Stem cells could be well differentiated cells with the potential to display different cell types depending on the host niche. Niche is defined as the cellular microenvironment providing support and stimuli to control the properties of stem cells. It consists of signaling molecules, inter-cell contacts and interaction between stem cells and their extracellular matrix neighbors. Stem cells are classified according to their sources into two main types, the embryonic and non-embryonic. Embryonic stem cells are pluripotent and can differentiate into all germ layers. Non-embryonic stem cells can be sub-classified into fetal stem cells and adult stem cells. Cultured cells can be made to differentiate into exclusive lineages by providing selective media components that can be identified by histochemical staining and quantified by quantitative Real-time polymerase chain reaction. Mesenchymal stem cells (MSCs) can be identified based on the expression of specific proteins called surface antigen phenotype of mesenchymal stem cell markers. MSCs secrete a variety of interleukins, several neurotrophic factors, many cytokines, and growth factors. These secreted bioactive factors have both paracrine and autocrine effects, which are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis. Furthermore, they stimulate mitosis and differentiation of tissue-intrinsic reparative stem cells. Systemic MSC transplantation can engraft to an injured tissue and promote wound healing through differentiation, and proliferation in synergy with hematopoietic stem cells. MSCs have been shown to express a variety of chemokines and chemokine receptors and can home to sites of inflammation by migrating towards injury or inflammatory chemokines and cytokines. MSCs are proven to have immunomodulatory properties that are among the most intriguing aspects of their biology. The immunosuppressive properties of MSCs inhibit the immune response of naive and memory T cells in a mixed lymphocyte culture and induce mitogen. The systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders. MSCs have become an alternative source of cells that can be drawn from several these cells have been used as treatment to repair cartilage defects at early stages sources. Using the MSCs and directing them into chondrogenic differentiation might lead to the formation of higher quality cartilage, which has a great composition of hyaline, adequate structural reorganization and therefore improved biomechanical properties.

Conclusion: It can be concluded that stem cells are classified according to their sources into two main types, the embryonic and non-embryonic. Embryonic stem cells are pluripotent and can differentiate into all germ layers. Non-embryonic stem cells can be sub-classified into fetal stem cells and adult stem cells. MSCs secrete bioactive factors that are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis. The systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders. These cells have been used as treatment to repair cartilage defects at early stages.

Keywords: stem cells, niche, classification, identification, characterization, applications in joint disease remedy.

1. Introduction

Stem cells are defined as cells that can self-renew indefinitely (dividing continuously). These divisions are asymmetric. One of the two daughter cells retains the stem cell characteristics, while the other is destined for a limited number of future divisions and will produce a more organ-specific cells) and able to differentiate into various cells when induced appropriately [1]. These cells have many other properties bringing to attention its application in regenerative medicine. Stem cell therapy has attracted much interest in the 21st century, not only because of the

controversy surrounding the ethics involving pluripotent stem cells, but their potential for clinical use [2].

2. Objectives

The present review highlights the stem cells niche, types, identification, and characterization, mechanisms of regeneration by using stem cells, and applications in joint disease remedy.

3. Niche of Stem Cells

Current research is focused on the microenvironment (niche) of stem cells, thus bringing several ideas that provide explanations for the stem cell line and determination of fate. A niche consists of signaling molecules, inter-cell contacts and interaction between stem cells and their extracellular matrix neighbors. The three-dimensional microenvironment is thought to control gene and properties that define the stemness, i.e., its self-renewal and development to committed cells [3]. Further studies of the niche may provide us with more information and a better understanding of the control of stem cell differentiation. Stem cells could be well differentiated cells with the potential to display different cell types depending on the host niche. In addition, stem cells located in completely different niches have the potential to differentiate into different cell types of the new environment [4]. These findings showed possible niche influence and the ability to dedifferentiate into cells from other lineages [5]. This may have clinical implications, such as neural stem cells produced when muscle cells were implanted in skeletal muscle [6] and bone marrow cells into neuronal cells when they were transplanted into an environment of neurons [7, 8]. As well as the liver and pancreas which develop from the same embryological line, specific growth factors and culture techniques can ensure the trans-differentiation of liver cells to islet cells [5]. Overall, Niche is defined as the cellular microenvironment providing support and stimuli to control the properties of stem cells. Further studies of the niche may provide us with more information and a better understanding of the control of stem cell differentiation and other cells trans-differentiation [4].

4. Classification of Stem cells

Nowadays, there are many bases to classified stem cells [9] for example, but not limited to

4.1 Classification of stem cells on the basis of potency:

Stem cells can be classified by the extent to which they can differentiate into different cell types [10, 11]:

1) **Totipotential stem cells:** The ability to differentiate into all possible cell types (form the embryo cells & extra embryo cells). Examples are the zygote formed at egg fertilization and the first few cells that result from the division of the zygote [12, 13].

2) **Pluripotential stem cells:** The ability to differentiate into almost all cell types. Examples include embryonic stem cells and cells that are derived from the mesoderm, endoderm, and ectoderm germ layers that are formed in the beginning stages of embryonic stem cell differentiation [12, 14].

3) **Multipotential stem cells:** The ability to differentiate into a closely related family of cells. Examples include hematopoietic, (adult) stem cells that can become red and white blood cell and platelets [12, 15, 16].

4) **Oligopotential stem cells:** The ability to differentiate into a few cells. Examples include (adult) lymphoid or myeloid stem cells [12, 17].

5) **Unipotential stem cells:** The ability to only produce cells of their own type, but have the property of self-renewal required to be labeled a stem cell. Examples include (adult) muscle stem cells [12, 15, 18].

4.2 Classification of stem cells on the basis of their sources [19]:

1) **Embryonic stem cells:** Embryonic stem cells are self-replicating pluripotent cells that are potentially immortal. They are derived from embryos at a developmental stage before the time of implantation would normally occur in the uterus. The embryos from which human embryonic stem cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst [20, 21].

2) **Non-Embryonic (Adult) stem cells:** Adult stem cells are undifferentiated multipotent cells, found throughout the body after embryonic development that multiply by cell division to replenish dying cells and regenerate damaged tissues. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. The origin of adult stem cells in some mature tissues is still under investigation [22, 23].

3) **Cloning (nuclear transfer) stem cells:** Somatic cell nuclear transfer (SCNT) is a technique for cloning. The nucleus is removed from a healthy egg (ova). This egg becomes the host for a nucleus that is transplanted from another cell, such as a skin cell. Then, using electrical shock called Roslin technique [24] or chemical shock called Honolulu technique [25] to start cell division & development. The resulting embryo (zygote/fertilized ova) can be used to generate embryonic stem cells with a genetic match to the nucleus donor [26].

4) **Induce Pluripotent (reprogramming) stem cells:** Scientists have engineered stem cell, with properties similar to embryonic stem cells, these induced pluripotent stem cells (iPS cells) by reprogramming technology which manipulating the expression of certain genes with reprogramming factors (oct4, soxy2, klf4 and myc), 'reprogramming' somatic cells back to a pluripotent state **[27, 28]**.

4.3 Classification of stem cells on the basis of transplanted stem cells sources for purpose of therapy [29]:

1) **Autologous stem cells:** referring to collected and transfer of stem cells from one location to another for transplanted into the same patient [30].

2) **Syngeneic stem cells:** referring to collected and transfer of stem cells or tissue or organ from one location of the person's identical twin to transplanted into patient [31].

3) **Allogeneic stem cells:** referring to collected and transfer of stem cells from one location of a donor person to transplanted into another person in same species (patient belong to same species) [32].

4) **Xenogeneic stem cells:** referring to collected and transfer of stem cells from one location of a donor person to transplanted into another species (patient belong to other species) [33].

5. Identification and Characterization of Stem Cells

5.1 Stemness

Stemness refers to combines the ability of stem cells to perpetuate their lineage (Self renew), to give rise to differentiated cells, and to interact with their environment to maintain a balance between quiescence, proliferation, and regeneration [34].

5.2 Morphology

Mesenchymal stem cells (MSCs) have been shown to adhere to cell culture flask and exhibit fibroblastic-like shape. Many studies have been demonstrated the effects of different culture protocols on the cell phenotype. The reports show little and no significant differences among the cells isolated by any protocol [35-37].

5.3 Cell differentiation

Differentiation is the process by which matured cells change to a specialized type. During differentiation, certain genes are turned on and become activated while others are switched off and become inactivated; a complex process tightly regulated, resulting in cell development of specific structures, which perform certain functions. Cultured cells can be made to differentiate into exclusive lineages by providing selective media components that can be identified by histochemical staining and quantified by quantitative Real-time polymerase chain reaction (RT-PCR) [38]. The standard test to confirm the mesenchymal stem cells is differentiation of the cells into other specific cells such as osteoblasts, adipocytes, chondrocytes, myocytes and neurons. MSCS have been seen to even differentiate into neuron-like cells. The process of differentiation normally will occur with the aid of influencing factors and it is considered the test for multipotency of MSCs [39].

5.4 Immunophenotyping

Immunophenotyping is using a flow cytometry technique to enable identification of specific cell types from complex biological samples according to the cell surface antigen expression. Mesenchymal stem cells can be identified based on the expression of specific proteins called surface antigen phenotype of mesenchymal stem cell markers. Some of these markers are present on undifferentiated MSCs and disappear during differentiation [40]. Some of these surface antigens may be expressed on the other cells but by using a profile of positive phenotypes which include Stro-1 (Stromal marker) and cluster of differentiation (CD). The specific positive phenotypes include CDI0S (Endoglin) CD106 (VCAM-1), CD10 (Antibody of common acute lymphoblastic leukemia antigen: anti-CALLA), CD117 (Stem cell factor receptor), CD120a (TNF receptor Type I), CD120b (TNF receptor Type l), CD13 (lntegral membrane glyeoprotein), CD15 (SSEA-1,Lewis X), CD166 (ALCAM), CD271 (p57, NGFR/NTR), CD29 (Integrin bl), CD44 (Pgp-1, HCAM), CD49d (lntegrin alpha4), CD49e (Integrin alpha5), CD51(Integrin alpha V), CD71 (Tranferrin Receptor), CD73 (Ecto5' nucleotidase), CD90 (Thy-1), Flk-1 (KDR, VEGF-R2,LY-73), Ly-6A/E (Sca-1), (Intermediate filament in Vimentin CO-activator), TAZ (Transcriptional cytoskeleton) and CD14 (Monocyte or macrophage antigen). On the other hand, the negative phenotypes include CDIla (Integrin al chain), CDI 1b (Integrin alpha M chain), CD31 (PECAMI), CD34 (Mucosialin, gp 105-120) and CD45 (Leukocyte Common Antigen, Ly-5). As can be recalled, it is possible to segregate specific type of cells based on the above-mentioned phenotypes. Many studies have used this experimental technique to identify and separate the stem cells from bone marrow cells as well as tissue derived cells [41-43].

6. Mechanisms of Regeneration by Using Stem Cells

6.1 Stemness

Stemness encompasses the capability of stem cells for self-renewal and differentiation to exhibit the potential to differentiate into various lineages of cells [44]. In other words, it refers to the capacity of stem cells to self-renew and give rise to progeny capable of differentiating into diverse cell types [45].

6.2 Trophic Support

MSCs secrete a variety of interleukins such as: (L-3, IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, and IL-15). Also, they secret several neurotrophic factors such as: Nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF), and Glial cell- derived neurotrophic factor (GDNF). Besides that, they secret many cytokines and growth factors such as: Leukemia inhibitory factor (LIF), Vascular endothelial growth factor (VEGF), Hepatocyte growth factor (HGE), Stromal derived factor (SDF), Stem Cell Factor (SCF), Macrophage-colony stimulating factor (M-CSF), Basic fibroblast growth factor (bFGF), insulin-like growth factor binding protein (IGFBP), Oncostatin M (OSM), Macrophage inflammatory protein-1ß (MIP-1B), tissue inhibitor of metalloproteinase: (TIMP-1 & TIMP-2), Transforming growth factor beta: (TGF-B1 & TGF-B 2), Platelet-derived growth factor (PDGF), Epidermal growth factor (EGF), and Keratinocyte Growth Factor (KGF) [46, 47]. These secreted bioactive factors have both paracrine and autocrine effects, which are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis [46, 48, 49]. Furthermore, they stimulate mitosis and differentiation of tissue-intrinsic reparative stem cells. These effects known as trophic effects are distinct from direct differentiation of MSCs in tissue repair. Several studies that tested the use of MSCs in different models such as myocardial infarction (heart injury), stroke (brain), or models of regeneration of the meniscus are reviewed in the context of MSC- mediated trophic effects in tissue repair [48].

6.3 Anti-inflammatory

MSCs have been proposed to have anti-inflammatory properties, which in recent years have generated much interest. The anti-inflammatory properties of MSCs are achieved through decrease or inhibition of pro-inflammatory mediators like IL-1, IL- 6, TNF-a, and IFN-y, as well as increase in anti-inflammatory mediators like IL-4, IL-10, IL-11 and IL-13. These effectively reduce inflammation. There are many studies that demonstrate the beneficial effects of MSC application for restoration of the stem cell niche in damaged tissues with its anti-inflammatory activity [50-53].

6.4 Differentiation into Tissue

MSCs are thought to be multipotent cells that mainly present in bone marrow (BM) [41, 54]. Many reports have indicated that MSCs that have the potential to differentiate into a wide range of tissues, including bone, cartilage, adipose tissue, muscle tissue and both in vivo and ex vivo [35, 55]. These adult stem cells could be induced to differentiate exclusively into the adipocytic, chondrocytic, or osteocytic lineages. Individual stem cells were identified that, when expanded to colonies, retained their multi-lineage potential. In response to experimental conditions, MSCs could also differentiate into cells of three germ layers [56, 57]. This type of cross-line age differentiation is known as trans-differentiation, which implies that adult stem cells contain multi-differentiation potentials. It has been shown in vivo that MSCs can generate epithelial cell types in skin, lung, and other tissues [58, 59]. Many studies suggest that systemic MSC transplantation can engraft to an injured tissue and promote wound healing through differentiation, proliferation, and in synergy with hematopoietic stem cells [60]. A recent report suggested that the transplantation of MSCs could rebuild damaged tissues [61].

6.5 Homing to Injury Site

The homing to injury or inflammation site is a unique property for MSCs that has sparked particular interest for clinical applications aiming at using noninvasive systemic cell administration to treat injury or inflammation.

MSCs have been shown to express a variety of chemokines and chemokine receptors and can home to sites of inflammation by migrating towards injury or inflammatory chemokines and cytokines [62, 63]. According to studies, the heterogeneity in the expression of surface receptors was observed, which is probably due to differences in growing conditions and limits of detection techniques, Homing of cultured MSCs, however, is inefficient compared with leukocytes. This inefficiency has been attributed to a lack of cell adhesion and chemokine receptors, but also to the size of MSCs that promote cellular passive trapping and reduce traffic [64]. Moreover, the evidence that host MSCs can mobilize in response to inflammation or injuries can be seen in the ability of systemically infused MSCs to be observed within the bone marrow or damaged tissues [65].

6.6 Immune System Modulation

MSCs are proven to have immunomodulatory properties that are among the most intriguing aspects of their biology [66]. As is widely known, the expression of major histocompatibility complex (MHC) molecules on all cells of the body allows the immune system to distinguish self from non-self [35, 41, 67-69]. Therefore, non-self-cells or tissues are rejected by the immune system through direct or indirect pathways. Cells expressing MHC molecules stimulate T cells directly only if they have the co-stimulatory molecules, or by activating T cells through an indirect pathway where their MHC antigens are presented by professional antigen presenting cells (APC). At the end, they will be rejected by immune defenses of the body through stimulating an immune response However, in contrast, undifferentiated MSCs fail to induce immune responses, even in the presence of APCs or after provision of co-stimulatory signals. This is because the immunosuppressive properties of MSCs inhibit the immune response of naive and memory T cells in a mixed lymphocyte culture (MLC) and induce mitogen [66, 70-78]. Previous studies showed that MSCs inhibit the division of stimulated T cells via preventing their entry into the S phase of the cell cycle and through mediating an irreversible G0/G1 phase arrest [79]. There are several contrasting studies that showed that MSCs not only appear to down-regulate the immune-reactivity of a variety of effector cells, but also escape immune rejection through escape lysis by CD8+ cytotoxic lymphocytes [66, 71, 80]. Indeed, there are reports suggesting that the systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders [65].

7. Applications of Mesenchymal Stem Cells in Joint Disease Remedy

Over the last decade, MSCs have become an alternative source of cells that can be drawn from several sources. These cells have been used as treatment to repair cartilage defects at early stages. The MSC-based cartilage repair has been attempted in animal models using various carrier matrices and synthetic polymers such as poly alpha-hydroxy esters (P a-hydroxy E), poly lactic acid (PLA), poly glycolic acid (PGA) and their copolymer poly lactic-co-glycolic acid (PLGA) [81-83]. Delivering stem cells by using techniques like micro-fracture are performed by penetration of the subchondral bone. When the tourniquet is released, the possible recruitment of stem cells from the underlying bone marrow leads to the formation of a super clot [84, 85]. Report showed 11% of biopsies being predominantly hyaline cartilage and 17% of a mixture of fibrocartilage and hyaline [86]. However, this technique is not sufficient for large lesions and the results have not always been consistent [87]. Using the MSCs and directing them into chondrogenic differentiation might lead to the formation of higher quality cartilage, which has a great composition of hyaline, adequate structural reorganization and therefore improved biomechanical properties. Wakitani *et al*. [88] successfully used MSCs gel type I collagen to repair chondral o caprine model and subsequently translated it into clinical practice. Autologous bone marrow derived MSC transplantation has been used to repair full-thickness defects of articular cartilage in the knee caps of two patients [89]. Wakitani *et al.* [90] also reported on twelve patients suffering from knee OA (Osteoarthritis) who received MSCs injected into cartilage defects of the medial femoral condyle at the time of high tibial osteotomy. These were then covered by periosteum. Although the clinical improvement was not significantly different, MSCs treated patients had better arthroscopic and histological grading scores. In rabbit model, MSCs have been cultured then transplanted into defective cartilages, which have been created in the left medial femoral condyle. The repair tissue approximated intact cartilage and was Superior to osteo-chondral auto-grafts and repair by innate mechanisms [91]. These findings suggest that repair of cartilage defects in the rabbit can be enhanced by the implantation of cultured MSCs [92]. In the near future, a new approach for OA could be the use of MSCs to inhibit disease progression. In OA, it has been demonstrated that stem cells are depleted and/or have reduced proliferation and differentiation capacity [93]. Thus, systemic or local administration of stem cells could increase the population of regenerative cells and possibly induce repair or inhibit the progression of the disease. Local delivery of adult MSCs suspended in sodium hyaluronan into injured joints of a caprine OA model showed that. the MSCs stimulated regeneration off meniscal tissue. Degenerated cartilage, osteophytic remodeling, and subchondral sclerosis were reduced in the cell-treated joints compared to controls [94]. These experiments implicate that MSCs hold exciting promise for regenerating meniscus and preventing OA. A group of researchers from Singapore have used this procedure clinically, with promising results [8]. In a sheep model of OA treated with autologous BMSCs cultured in chondrogenic medium, there was Clear evidence of articular cartilage regeneration [95].

8. Conclusion

It can be concluded that stem cells could be well differentiated cells with the potential to display different cell types depending on the host niche. Stem cells are classified according to their sources into two main types, the embryonic and non-embryonic. Embryonic stem cells are pluripotent and can differentiate into all germ layers. Non-embryonic stem cells can be sub-classified into fetal stem cells and adult stem cells. MSCs secrete bioactive factors that are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis. The systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders. These cells have been used as treatment to repair cartilage defects at early stages.

References

1. Gunnar, K., Lars, E., Tom, C., Jon Olav, D., Torbjorn, G., Eirik, S., et al. (2004). Autologous Chondrocyte Implantation Compared with Microfracture in the KneeA Randomized Trial. The Journal of Bone and Joint Surgery (American), 86(3): 455-464.
2. Im G. I., Kim, D. Y., Shin, J. H., Hyun, C. W., & Cho, W. H. (2001). Repair of cartilage defect in the rabbit with cultured mesenchymal stem cells from bone marrow. Journal of Bone and Joint Surgery-British Volume, 83(2): 289-294.
3. Watt, F. M., and Hogan, B. L. (2000). Out of Eden: stem cells and their niches. Science, 287(5457): 1427-1430.
4. Wu, P., Tarasenko, Y. I., Gu, Y., Huang, L. Y. M., Coggeshall, R. E., and Yu, Y. 2002).) Region-specific generation of cholinergic neurons from fetal human neural stem cells grafted in adult rat. Nature Neuroscience, 5(12), 1271-1278.
5. Alam, T., and Sollinger, H. W. (2002). Glucose-regulated insulin production in hepatocytes. Transplantation, 74(12), 1781-1787.
6. Ghannam, S., Bouffi, C Djouad, F., Jorgensen, C., and Noel, D. (2010). Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Sfem Cell Kesearch and Therapy, I(1): 1-7..
7. Zhao, L. R. Duan, W. M., Reyes, M., Keene, C. D., Verfaillie, C. M., and Low, W. C. (2002). Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits

after grafting into the ischemic brain of rats .Experimental Neurology, 174(1): 11-20.
8. Rajada. S., Mazakova, I., Richardson, J. B., and Ashammakhi, N. (2008). Updates on stem cells and their applications in regenerative medicine. Journal of Tissue Engineering and Regenerative Medicine, 2(4): 169-183.
9. Gehart, H. and H. Clevers (2019). "Tales from the crypt: new insights into intestinal stem cells." Nature Reviews Gastroenterology & Hepatology 16(1): 19-34.
10. Kalra, K. and P. Tomar (2014). "Stem cell: basics, classification and applications." American Journal of Phytomedicine and Clinical Therapeutics 2(7): 919-930.
11. Plass, M., Solana, J., Wolf, F. A., Ayoub, S., Misios, A., Glažar, P., and Rajewsky, N. (2018). Cell type atlas and lineage tree of a whole complex animal by single-cell transcriptomics. Science, 360(6391).
12. Alison, M. R., Poulsom, R., Forbes, S., and Wright, N. A. (2002). "An introduction to stem cells." The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland 197(4): 419-423.
13. Baker, C. L. and Pera M. F. (2018). "Capturing totipotent stem cells." Cell Stem Cell 22(1): 25-34.
14. Yu, J. and J. A. Thomson (2008). Pluripotent stem cell lines. Genes and development 22(15): 1987-1997.
15. Corley, L. S. and Lavine M. D.(2006). A review of insect stem cell types. Seminars in cell & developmental biology, Elsevier.
16. Sobhani, A., Khanlarkhani, N., Baazm, M., Mohammadzadeh, F., Najafi, A., Mehdinejadiani, S., and Aval, F. S. (2017). "Multipotent stem cell and current application." Acta Medica Iranica: 6-23.
17. Majo, F., Rochat, A., Nicolas, M., Abou Jaoudé, G., and Barrandon, Y. (2008). Oligopotent stem cells are distributed throughout the mammalian ocular surface. Nature, 456(7219): 250-254.
18. Presnell, S. C., Petersen, B., and Heidaran, M. (2002). Stem cells in adult tissues. In Seminars in cell & developmental biology, 13(5): 369-376.
19. Rao, M. S. (2004). Stem sense: a proposal for the classification of stem cells. Stem cells and development, 13(5): 452-455.
20. Wobus, A. M. (2001). Potential of embryonic stem cells. Molecular aspects of medicine, 22(3): 149-164.
21. Martello, G. and A. Smith (2014). The nature of embryonic stem cells. Annual review of cell and developmental biology 30.
22. Young, H. E. and A. C. Black Jr (2004). Adult stem cells. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology: An Official Publication of the American Association of Anatomists 276(1): 75-102.
23. Alison, M. and S. Islam. (2009). Attributes of adult stem cells. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland 217(2): 144-160.
24. Butler, D. (1997). Roslin patents come under the spotlight, Nature Publishing Group.
25. Watts, J. and K. Morris (1999). Human cloning trial met with outrage and scepticism. The Lancet, 353(9146): 43.
26. Byrne, J. A., Pedersen, D. A., Clepper, L. L., Nelson, M., Sanger, W. G., Gokhale, S., and Mitalipov, S. M. (2007). Producing primate embryonic stem cells by somatic cell nuclear transfer. Nature 450(7169): 497-502.
27. Takahashi, K. and Yamanaka S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4): 663-676.
28. González, F., Boué, S., & Belmonte, J. C. I. (2011). Methods for making induced pluripotent stem cells: reprogramming a la carte. Nature Reviews Genetics 12(4): 231-242.
29. Fortier, L. A. (2005). Stem cells: classifications, controversies, and clinical applications. Veterinary Surgery, 34(5): 415-423.
30. Prasongchean, W. and Ferretti P. (2012). Autologous stem cells for personalised medicine. New biotechnology, 29(6): 641-650.
31. Yoshida, S., Miyagawa, S., Toyofuku, T., Fukushima, S., Kawamura, T., Kawamura, A., and Sawa, Y. (2020). Syngeneic mesenchymal stem cells reduce immune rejection after induced pluripotent stem cell-derived allogeneic cardiomyocyte transplantation. Scientific reports, 10(1): 1-11.
32. Singh, A. K. and J. P. McGuirk (2016). Allogeneic stem cell transplantation: a historical and scientific overview. Cancer research, 76(22): 6445-6451.
33. Yang, Y. G. (2004). Application of xenogeneic stem cells for induction of transplantation tolerance: present state and future directions. In Springer seminars in immunopathology, 26 (1-2): 187-200.
34. Aponte, P. M. and A. Caicedo (2017). Stemness in cancer: stem cells, cancer stem cells, and their microenvironment. Stem Cells International 2017.
35. Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D. et al. (1999). Multilineage potential of adult human mesenchymal stem cells. Science, 284(5411): 143-147.
36. Lodie, T. A., Blickarz, C. E., Devarakonda, T. J., He, C., Dash, A. B., Clarke, J., et al. (2002). Systematic analysis of reportedly distinct populations of multipotent bone marrow-derived stem cells reveals a lack of distinction. Tissue Engineering, 8(5): 739-751.
37. Conget, P. A., and Minguell, J. J. (1999). Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. Journal of Cellular Physiology, 181(1): 67-73.
38. Banfi, A., Muraglia, A., Dozin, B., Mastrogiacomo, M., Cancedda, R., and Quarto, R. (2000). Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. Experimental Hematology, 28(6): 707-715.
39. Jiang, Y.. Vaessen, B., Lenvik, T., Blackstad, M., Reyes, M., & Verfaillie, C. M. (2002). Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Experimental Hematology, 30(8): 896-904.
40. Simmons, P. J., and Torok-Storb, B. (1991). Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. Blood, 78(1): 55-62
41. Majumdar, M. K., Thiede, M. A., Mosca, J. D., Moorman, M., and Gerson, S. L. (1998). Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. Journal of Cellular Physiology, 176(1): 57-66.
42. Arai, F., Ohneda, O., Miyamoto, T., Zhang, X. Q., and Suda, T. (2002). Mesenchymal stem cells in perichondrium express activated leukocyte cell adhesion molecule and participate in bone marrow formation. The Journal of Experimental Medicine, 195(12): 1549-1563.
43. Shur, I., Marom, R., Lokiec, F., Socher, R., and Benayahu, D. (2002). Identification of cultured progenitor cells from human marrow stroma. Journal of Cellular Biochemistry, 87(1), 51-57.
44. Melton, D. (2014). 'Stemness': definitions, criteria, and standards. Essentials of stem cell biology, Elsevier: 7-17.
45. Wu, J. and Belmonte J. C. I. (2016). Stem cells: a renaissance in human biology research. Cell 165(7): 1572-1585.
46. Rehman, J., Traktuev, D., Li, J., Merfeld- Clauss, S., Temm-Grove, CJ, Bovenkerk, J.E., Pell, CL, Johnstone, BH, Considine, RV, and March, KL. (2004). Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation, (109): 1292-1298.

47. Tschope, C., Miteva, K., Schultheiss, H. P., and Van Linthout, S. (2011). Mesenchymal Stromal Cells: A Promising Cell Source for the Treatment of Inflammatory Cardiomyopathy. Current Pharmaceutical Design, 17(30): 3295-3307..
48. Caplan, A. I., and Dennis, J. E. (2006). Mesenchymal stem cells as trophic mediators. Journal of Cellular Biochemistry, 98(5): 1076-1084.
49. Nakagami H, M. R., Maeda K, Kikuchi Y, Ogihara T, and Kaneda Y. (2006). Adipose Tissue-Derived Stromal Cells as a Novel Option for Regenerative Cell Therapy. Journal of Atherosclerosis and Thrombosis, 13(2): 77-81.
50. Le Blanc, K., Rasmusson, I., Götherström, C., Seidel, C., Sundberg, B., Sundin, M., et al. (2004). Mesenchymal Stem Cells Inhibit the Expression of CD25 (Interleukin-2 Receptor) and CD38 on Phytohaemagglutinin-Activated Lymphocytes. Scandinavian Journal of Immunology, 60(3): 307-315.
51. Le Blanc, K., and Ringden, O. (2007). Immunomodulation by mesenchymal stem cells and clinical experience. Journal of Internal Medicine, 262(5): 509-525.
52. Iyer, S. S., & Rojas, M. (2008). Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies. Expert opinion on biological therapy, 8(5), 569-581.
53. Uccelli, A., Moretta, L., and Pistoia, V. (2008). Mesenchymal stem cells in health and disease. Nature Reviews Immunology, 8(9): 726-736.
54. Tuli, R., Seghatoleslami, M. R., Tuli, S., Wang, M. L., Hozack, W. J., Manner, P. A et al. (2003). A simple, high-yield method for obtaining multipotential mesenchymal Molecular cells from trabecular bone. Progenitor Biotechnology, 23(1): 37-49.
55. Prockop. D. J. (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. Science, 276(5309): 71-74.
56. Kopen, G. C., Prockop, D. J., and Phinney, D. G. (1999). Marrow stromal cells grate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proceedings of the National Academy of Sciences, 96(19): 10711-10716.
57. Liechty, K. W., MacKenzie, T. C., Shaaban, A. F., Radu, A., Moseley, A. M. B., Deans, R., et al. (2000). Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nature Medicine, 6(11), 1282-1286.
58. Kotton, D. N., Ma, B. Y., Cardoso, W. V., Sanderson, E. A., Summer, R. S. Williams, M. C., et al. (2001). Bone marrow-derived cells as progenitors of lung alveolar epithelium. Development, 128(24), 5181-5188.
59. Ye, J., Yao, K., and Kim, J. (2005). Mesenchymal stem cell transplantation in a rabbit corneal alkali burn model: engraftment and involvement in wound healing. Eye, 20(4): 482-490.
60. Ma, Y. Xu, Y., Xiao, Z., Yang, W., Zhang, C., Song, E., et al. (2006). Reconstruction of chemically burned rat corneal surface by bone marrow derived human mesenchymal stem cells. Stem Cells, 24(2): 315-321.
61. Sordi, V., Malosio, M. L., Marchesi, F., Mercalli, A., Melzi, R., Giordano, T., et al. (2005). Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. Blood, 106(2): 419-427.
62. Hui, J., Azura, M., and Lee, E. (2009). Review Article: Stem Cell Therapy in Orthopacdic Surgery: CurTent Status and Ethical Considerations. Malaysian Orthopaedic Journal 3(1): 4-12.
63. Karp, J. M., and Leng Teo, G. S. (2009). Mesenchyvmal stem cell homing: the devil is in the details. Stem Cell, 4(3): 206-216.
64. Glennie, S., Soeiro, I., Dyson, P. J., Lam, E. W. F., and Dazzi, F. (2005). Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood, 105(7): 2821-2827.
65. Le Blanc, K., Tammik, C., Rosendahl, K., Zetterberg, E., and Ringden, O. (2003). Expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells, Experimental Hematology, 31(10),890-896.
66. De Ugarte, D. A., Alfonso, Z., Zuk, P. A., Elbarbary, A., Zhu, M., Ashjian, P., et al. (2003). Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. Immunology Letters, 89(2-3): 267-270.
67. Di Nicola, M., Carlo-Stella, C., Magni, M., Milanesi, M., Longoni, P. D., Matteucci, P., et al. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood, 99(10): 3838-3843.
68. Deans, R. J., & Moseley, A. B. (2000). Mesenchymal stem cells: Biology and potential clinical uses. Experimental Hematology, 28(8): 875-884.
69. Bartholomew, A., Sturgeon, Cs S1atskas, M., Ferrer, K., Mclntosh, K., Patil, S.. et al (2002). Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin gralt survival in vivo. Experimental Hematology, 30(1): 42- 48.
70. Galli, R., Borello, U., Gritti, A., Minasi, M. G., Bjornson, C., Coletta, M., et al. (2000). Skeletal myogenic potential of human and mouse neural stem cells.Nature Neuroscience, 3(10): 986-991.
71. Krampera, M., Glennie, S., Dyson, J., Scott, D., Laylor, R., Simpson, E., et al .(2003) .Bone marrow mesenchymal stem cells inhibit the response of naïve and memory antigen-specific T cells to their cognate peptide. Blood, 101(9): 3722-3729..
72. Potian, J. A., Aviv, H., Ponzio, N. M., HarTISon, J. S., and Rameshwar, P. (2003). Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. The Journal of Immunology, 171(7): 3426-3434.
73. Rasmusson, I., Ringdén, O., Sundberg, B., and Le Blanc, K. (2003). Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation, 76(8), 1208-1213.
74. Tse, W. T., Pendleton, J. D., Beyer, W. M., Egalka, M. C., and Guinan, E. C. (2003). Suppression of allogeneic I-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation, 75(3), 389-397.
75. Angoulvant, D., Clerc, A., Benchalal, S., Galambrun, C., Farre, A., Bertrand, Y., et al. (2004). Human mesenchymal stem cells suppress induction of cytotoxic response to alloantigens. Biorheology, 41(3-4): 469-478.
76. Maitra, B., Szekely, E., Gjini, K., Laughlin, M., Dennis, J., Haynesworth, S., et al.(2004). Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. Bone Marrow Transplantation, 33(6): 597-604
77. klyushnenkova, E., Mosca, J. D., Zernetkina, V., Majumdar, M. K., Beggs, K. J, Simonetti, D. W., et al. (2005). T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. Journal of Biomedical Science, 12(1): 47-57.
78. Rasmusson, I., Ringdén, O., Sundberg, B., and Le Blanc, K. (2005). Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Experimental cell research, 305(1): 33-41.

79. Guenechea, G., Gan, 0. I, Dorrell, C., and Dick, J. E. (2001). Distinct classes of human stem cells that differ in proliferative and self-renewal potential. Nature Immunolog, 2(0): 75-82.
80. Tse, W. T., Pendleton, J. D., Beyer, W. M., Egallka, M. C., and Guinan, E. C. (2003). Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. TIransplantation, 75(3), 389-397.
81. Peretti, G. M., Xu, J. W., Bonassar, L. J., Kirchhoff, C. H., Yaremchuk, M. J, and Randolph, M. A. (2006). Review of injectable cartilage engineering using fibrin gel in mice and swine models. Tissue Engineering 12(5): 1151-1168.
82. Shangkai, C., Naohide, T, Koji. Y., Yasuji, H, Masaaki, N., Tomohiro, T., et al. (2007). Transplantation of allogeneie chondrocytes cultured in fibroin sponge and stirring chamber to promote cartilage regeneration. Tissue Engineering, 13(3), 483-492.
83. Zwingmann, J., Mehlhorn, A. T., Südkamp, N., Stark, B., Dauner, M., and Schmal, H. (2007). Chondrogenic differentiation of human articular chondrocytes differs in biodegradable PGA/PLA scaffolds. Tissue engineering, 13(9): 2335-2343.
84. Steadman. J. R., Rodkey W. ngieton, S. B., and Briggs, K. K. (1997) Microfracture technique forfull-thickness chondral defects: Technique and clinical results. Operalive Techniques in Orthopaedics, 7(4): 300-304
85. Steadman, J. R., Rodkey, W. , and Kodig0, J. J. (2001). Microfracture: surgical technique and rehabilitation to treat chondral defects. Clinical Orthopedics and Related Research, 391, S362-S369.
86. Honczarenko, M., Le, Y., Swierkowski, M., Ghiran, I., Glodek, A. M., and Silberstein, L. E. (2006). Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. Stem Cells, 24(4): 1030-1041.
87. Kreuz, P., Steinwachs, M., Erggelet, C., Krause, S., Konrad, G., Uhl, M., et al (2006). Results after microfracture of full-thickness chondral defects in different compartments in the knee. Osteoarthritis and Cartilage, 14(11): 1119-1125.
88. Wakitani, S., Goto, T., Pineda, S. J., Young, R. G., Mansour, J. M., Caplan, A. I., and Goldberg, V. M. (1994). Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. The Journal of bone and joint surgery. American volume, 76(4): 579-592.
89. Wakitani, S., Mitsuoka, T., Nakamura, N., Toritsuka, Y., Nakamura, Y., and Horibe, S. (2004). Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. Cell Transplantation, 13(5): 595-600.
90. Wakitani, S., Imoto, K., Yamamoto, T., Saito, M., Murata, N., and Yoneda, M. (2002). Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis and Cartilage, 10(3): 199-206.
91. Nathan, S., De, S. D., Thambyah, A., Fen, C., Goh, J., and Lee, E. H. (2003). Cell- based therapy in the repair of osteochondral defects: a novel use for adipose tissue. Tissue Engineering, 9(4): 733-744.
92. Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L. Schwartz, R. E., Keene, C. D., Ortiz-Gonzalez, X. R., et al. (2002). Pluripotency of mescenchymal stem cells derived from adult marrow. Nature, 418(6893): 41-49.
93. Murphy, J. M., Dixon, K., Beck, S., Fabian, D., Feldman, A., and Barry, F. (2002). Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. Arthritis and Rheumatism, 46(3): 704-713.
94. Murphy, J. M., Fink, D. J., Hunziker, E. B., and Barry, F. P. (2003). Stem cell therapy in a caprine model of osteoarthritis. Arthritis and Rheumatism, 48(12), 3464- 3474
95. Alfaqeh, H., Norhamdan, M., Chua, K., Chen, Aminuddin, B., and Ruszymah, B. (2008). Cell based therapy for osteoarthritis in a sheep model: gross and histological assessment. The Medical Journal of Malaysia, 63, 37-38.