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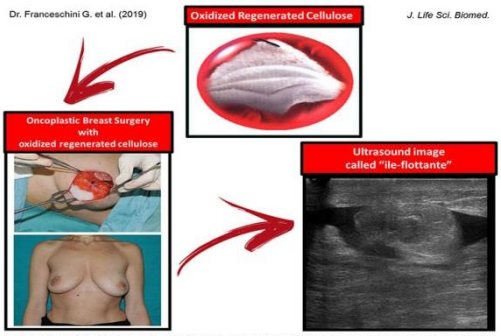
Volume 9 (2); March 25, 2019

Letter to Editor



Franceschini G, Di Leone A, Visconti G, Masetti R. 2019. **The use of a new hemostatic preparation made of the cellulose derivatives in surgery: "warning" for postoperative complications!** *J. Life Sci Biomed,* 9(2): 42-44; www.jlsb.science-line.com

The use of a new hemostatic preparation made of the cellulose derivatives in surgery: "warning" for postoperative complications!

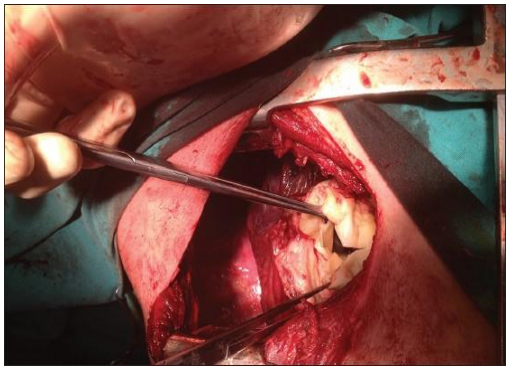
Franceschini G, Di Leone A, Visconti G, Masetti R.
J. Life Sci. Biomed., 9(2): 42-44, 2019;
pii:S225199391900007-9

ABSTRACT
Introduction. We have read with interest the article by Rustam Abrarovich Sadykov et al. (2019) on "New hemostatic preparation made of the cellulose derivatives". The Authors present their early experience on new samples of pellicle hemostatic coverage on the basis of the cellulose derivatives. They conclude: "Rapid enough biodegradation of polymer along with the unexpressed inflammatory reaction allows preventing the infecting related to the presence of foreign body. The rapid forming of fibrotic tissue in a zone of lesion makes it possible to obtain a durable hemostasis". **Results.** In our series we noted a 10% rate of allergic skin reactions with irritation, redness, itching, swelling, rash and hives in the mammary region, successfully managed with steroids and antihistamine medications. In addition, we experienced a significant seroma in the site of oxidized regenerated cellulose (ORC) placement in 45% of our patients. **Conclusion and Recommendation.** When using a new preparation made of the cellulose derivatives, as a possible aid to reduce the risk of postoperative haematoma and infections it is important to discuss with the patient also about possible postoperative complications. It is also important that surgeons specify clearly the use of this biomaterial in the report of the surgical procedure so that radiologists can properly interpret the sonographic findings due to this biomaterial and avoid misdiagnosis and undue alarmism during the follow-up of these patients.

Keywords: Hemostasis, Oxidized Cellulose, Polymer

[Full text-PDF] [XML]

Research Paper



Geprotsel, biocompatible implant: comparative estimation of its application results for providing airstasis and hemostasis in the lung surgery.

Khudaybergenov ShN, Eshonkhodjaev OD, and Khalmuratova MK.
J. Life Sci. Biomed., 9(2): 45-51, 2019;
pii:S225199391900008-9

ABSTRACT
Introduction. In surgery, the prevention of postoperative complications has always been and remains relevant. One of the most important components that contribute to reducing the number of complications, in addition to effective drainage, restoration of muscle tone and adequate breathing, is reliable aerostasis and hemostasis. When performing operations on the lungs against the background of the presence in patients of factors affecting the incidence of failure in aero- and hemostasis (COPD, emphysema), the risk of developing these complications can reach 11.8% after lobectomy, after wedge-shaped resections up to 9.1% and after decortication up to 33.3%, which is 14.7% for all operations in general (violation of aerostasis - 5.9% and hemostasis - 8.8%. **Aim.** The aim of study was to investigate the effectiveness of the proposed domestic implant "Geprocel" in the treatment and prevention of disorders of aero- and hemostasis during pulmonary operations. **Methods.** The study included 69 patients operated in the department of surgery of the Lung and Mediastinum of the "Republican Specialized Scientific and Practical Medical Center of Surgery named after Academician V. Vakhidov" State Institution for the period from 2015 to June 2018. Hemostatic implant in the form of a fine powder was developed at RSRCS named after acad. V. Vakhidov". Geprotsel consists of the following components: the sodium salt of carboxymethyl cellulose, oxidized cellulose and nanocellulose associated with calcium ions (Patent No. IAP 20160273), in accordance with requirements of ISO 10993-1-2011. **Results.** The use of the Heprotsel biological implant reduced the need for additional single lung tissue flashing to ensure adequate aero- and hemostasis from 38.2% to 11.4% and multiple reinforcement with sutures from 29.4% to 5.7% ($\chi 2 = 7.706$; Df = 2; P = 0.021).

Keywords: Aerostasis, Hemostasis, Collagen, Oxidized cellulose, Biodegradable implant, Geprotsel, Heprocel, Pulmonary operations
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Review

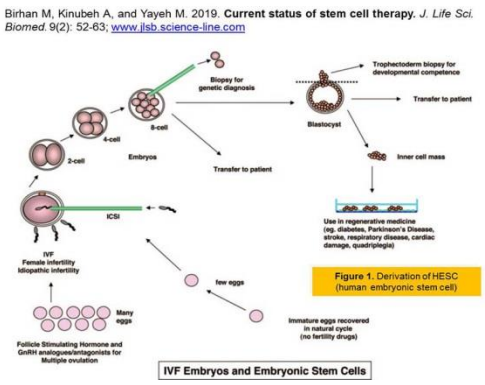

Figure 1. Derivation of HESC (human embryonic stem cell)

Current status of stem cell therapy.

Birhan M, Kinubeh A, and Yayeh M.

J. Life Sci. Biomed., 9(2): 52-63, 2019;
pii:S225199391900009-9

ABSTRACT

Introduction. Stem cells have the extraordinary potential to develop into many diverse cell types in the body during early life and growth. Significant progress has been made in understanding the biochemical and metabolic mechanisms and feedback associated with different stem cells response. Some of the challenges concerning transplanted embryonic stem cells and mesenchymal stem cells are immune-mediated rejection, senescence-induced genetic instability or loss of function, and limited cell survival. **Aim.** The aim of this review, is to recapitulate the recent status and information about the use of embryonic stem cells and mesenchymal stem cells for research into how cells and tissues of the body grow and develop, and potentially useful for curing disease. **Results.** Stem cell therapy efforts are currently underway for virtually every type of tissue and organ within the human body. Because the current status of stem cell incorporates the fields of cell transplantation, materials science, and engineering, personnel who have mastered the techniques of cell harvest, culture, expansion, transplantation, and polymer design are essential for the successful application of this technology. Various stem cell therapies are at different stages of development, with some already being used clinically, a few in preclinical trials, and some in the discovery stage. **Recommendations.** Recent progresses suggest that stem cell therapy may have expanded clinical applicability in the future because they represent a viable therapeutic option for those who require tissue and cells replacement in diverse degenerative disease. More recently, major advances in the areas of stem cell biology, tissue engineering, and nuclear transfer techniques have made it possible to combine these technologies to create the comprehensive scientific field of regenerative medicine. "But there is a strong need for better understanding the biology, manipulation and safety of stem cells in tissue regeneration and repair before starting the therapeutic applications."

Keywords: Embryonic Stem Cell, Mesenchymal Stem Cell, Regenerative

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The use of a new hemostatic preparation made of the cellulose derivatives in surgery: "warning" for postoperative complications!

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ABSTRACT

Introduction. We have read with interest the article by Rustam Abrarovich Sadykov et al. (2019) on "New hemostatic preparation made of the cellulose derivatives" [1]. The Authors present their early experience on new samples of pellicle hemostatic coverage on the basis of the cellulose derivatives. They conclude: "Rapid enough biodegradation of polymer along with the unexpressed inflammatory reaction allows preventing the infecting related to the presence of foreign body. The rapid forming of fibrotic tissue in a zone of lesion makes it possible to obtain a durable hemostasis".

Results. In our series we noted a 10% rate of allergic skin reactions with irritation, redness, itching, swelling, rash and hives in the mammary region, successfully managed with steroids and antihistamine medications. In addition, we experienced a significant seroma in the site of oxidized regenerated cellulose (ORC) placement in 45% of our patients.

Conclusion and Recommendation. When using a new preparation made of the cellulose derivatives, as a possible aid to reduce the risk of postoperative haematoma and infections it is important to discuss with the patient also about possible postoperative complications. It is also important that surgeons specify clearly the use of this biomaterial in the report of the surgical procedure so that radiologists can properly interpret the sonographic findings due to this biomaterial and avoid misdiagnosis and undue alarmism during the follow-up of these patients.

Letter to Editor

PII: S225199391900007-9

Rec. 18 February 2019
Rev. 23 March 2019
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Keywords

Hemostasis,
Oxidized Cellulose,
Polymer

DISCUSSION

We have previously reported our experience with the use of oxidized regenerated cellulose (ORC), at the Catholic Breast Unit of Rome, as a possible aid to reduce the risk of postoperative haematoma and infections and to improve the aesthetic outcomes in patients undergoing an oncoplastic procedures for breast cancer [2, 3].

However, as new hemostatic preparations made of the cellulose derivatives is being increasingly utilized in surgery [1-6], we think that it is important to properly inform the patients not only about the potential advantages but also about possible postoperative complications of these materials. Tanaka et al. [4] report a 18% rate of allergic reaction with the use of ORC, mainly presenting as acute dermatitis and eczema, and one case of exudation followed by wound dehiscence [4].

In our series we noted a 10% rate of allergic skin reactions with irritation, redness, itching, swelling, rash and hives in the mammary region, successfully managed with steroids and antihistamine medications. In addition, we experienced a significant seroma in the site of ORC placement in 45% of our patients [3]. This seroma, that appears in the early postoperative period as consequence of redundant ORC digestion, normally resolved within few weeks with repeated percutaneous aspirations but in two cases it was followed by the

formation of an abscess in the residual cavity that required surgical drainage. We also had a case of a foreign body reaction that required surgical excision to solve the complication (Figure 1).

Besides, we think it is important to call the attention of radiologists on the peculiar findings that preparation made of the cellulose derivatives as ORC may determine on postoperative ultrasound (US) examination, that often lead to undue alarmism.

In our series, peculiar fluid anaechoic accumulation containing small hyperechoic, round components were documented on breast US examination (performed six months after surgery) in all cases. This typical round image (that we named "ile-flottante") (Figure 2), is consequence of the fibrogenetic action induced by ORC and of the partial reabsorption of this biomaterial. It appears non-mobile, avascular, and adherent to the parenchymal tissue planes and is often misinterpreted in an alarming way by the radiologists. The diagnostic interpretations in our patients varied from possible residual disease to haematoma sequaele, local abscess or area of fat necrosis.

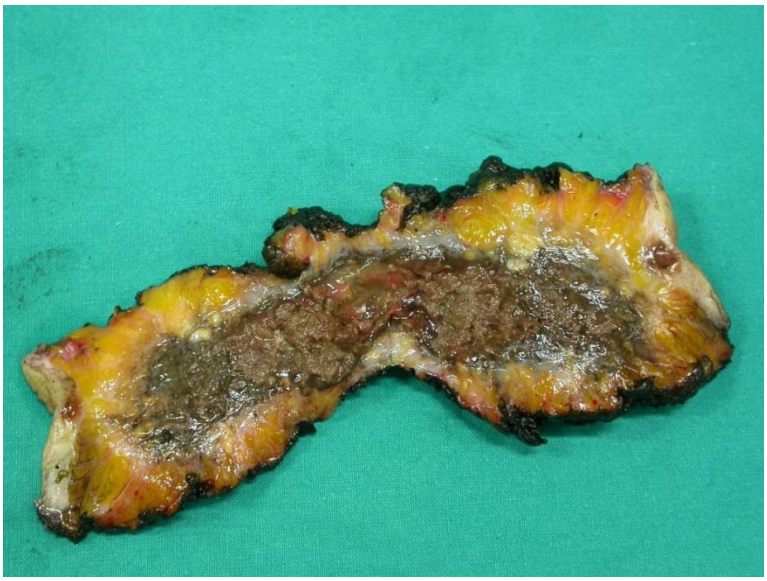

Figure 1. A foreign body reaction that required surgical excision after six-month follow-up in a patient treated by breast oncoplastic conservative surgery with ORC.

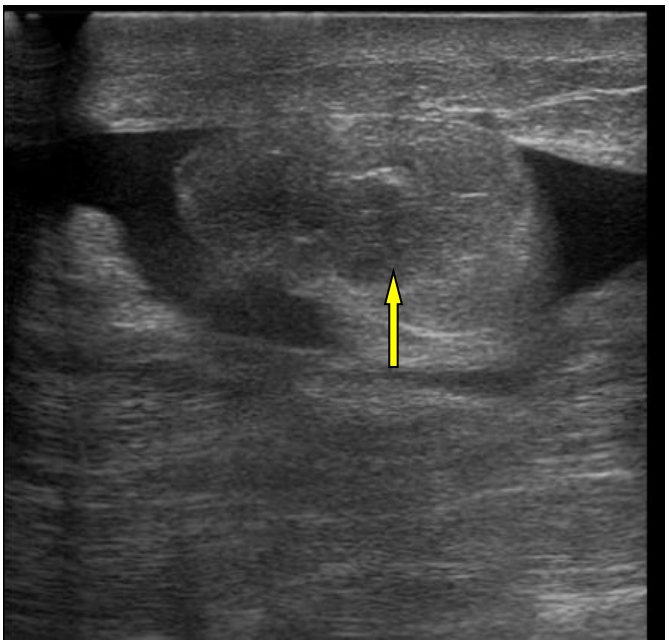

Figure 2. Ultrasound images (Siemens Antares sonography unit, Siemens Medical Solutions, Sweden) at six-month follow-up in three patients treated by breast oncoplastic conservative surgery with ORC. With the use of a high-frequency 10–13 MHz linear array transducer, a free anaechoic collection without wall with the presence of typical small hyperechoic round masses (yellow arrow) in continuity with the breast parenchyma is showed.

CONCLUSION

In conclusion, when using a new preparation made of the cellulose derivatives, as a possible aid to reduce the risk of postoperative haematoma and infections it is important to discuss with the patient also about possible postoperative complications. It is also important that surgeons specify clearly the use of this biomaterial in the report of the surgical procedure so that radiologists can properly interpret the sonographic findings due to this biomaterial and avoid misdiagnosis and undue alarmism during the follow-up of these patients.

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Geprotsel, biocompatible implant: comparative estimation of its application results for providing airstasis and hemostasis in the lung surgery

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ABSTRACT

Introduction. In surgery, the prevention of postoperative complications has always been and remains relevant. One of the most important components that contribute to reducing the number of complications, in addition to effective drainage, restoration of muscle tone and adequate breathing, is reliable aerostasis and hemostasis. When performing operations on the lungs against the background of the presence in patients of factors affecting the incidence of failure in aero- and hemostasis (COPD, emphysema), the risk of developing these complications can reach 11.8% after lobectomy, after wedge-shaped resections up to 9.1% and after decortication up to 33.3%, which is 14.7% for all operations in general (violation of aerostasis, 5.9% and hemostasis, 8.8%. Aim. The aim of study was to investigate the effectiveness of the proposed domestic implant "Geprocel" in the treatment and prevention of disorders of aero- and hemostasis during pulmonary operations. Methods. The study included 69 patients operated in the department of surgery of the Lung and Mediastinum of the "Republican Specialized Scientific and Practical Medical Center of Surgery named after Academician V. Vakhidov" State Institution for the period from 2015 to June 2018. Hemostatic implant in the form of a fine powder was developed at RSRCS named after acad. V. Vakhidov". Geprotsel consists of the following components: the sodium salt of carboxymethyl cellulose, oxidized cellulose and nanocellulose associated with calcium ions (Patent No. IAP 20160273), in accordance with requirements of ISO 10993-1-2011. Results. The use of the Heprotsel biological implant reduced the need for additional single lung tissue flashing to ensure adequate aero- and hemostasis from 38.2% to 11.4% and multiple reinforcement with sutures from 29.4% to 5.7% ($\chi 2 = 7.706$; Df = 2; P = 0.021).

Original Article

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Aerostasis,
Hemostasis,
Collagen,
Oxidized cellulose,
Biodegradable implant,
Geprotsel,
Heprocel,
Pulmonary operations

INTRODUCTION

The issues of postoperative complications still remain actual for the surgery. As it is known, the main predetermining moment in the prophylaxis of respiratory disorders and prevention of infectious complications at the thoracic surgeries is a quick and a complete spread of lung in the postoperative period. A reliable airstasis and hemostasis besides effective drainage, recovery of muscular tonus and adequate respiration are very important promoting factors [1-2].

The absence of persistent airstasis leads to: an incomplete spread of lung, a pneumothorax with a formation of residual cavities, the development of empyema and bronchial fistulas. Theses complications together with the infection become a main cause of progressing respiratory and cardiac failures leading to the lethal outcomes [3-4].

Unconvincing intraoperative air- and hemostasis and complications force sometimes to increase the scope of surgery; seal failure of the pleural cavity in the early postoperative period in some cases serves as indication for the rethoracotomy and the extension of surgery scope due to the remained lung lobes [5].

The problems connected with air- and hemostasis are one the most often occurred in the lung surgery. A variety of methods for solving them have been offered, but the majority of them are characterized by the prime cost of the used material. So, the development of domestic materials for their use at different surgical interventions, particularly in the lung surgery is an actual issue of health care. In our previous researches, we proved the efficiency of proposed biodegradable polycomposite implant with oxidized cellulose – "Geprotsel"

with air- and hemostatic aim at lung surgeries. Subject to the positive results of experimental investigations the next stage for the biological implant efficiency were clinical trials.

Polymer implants are increasingly used in medicine. Cellulose derivates are non-toxic, have good biocompatibility and provide tremendous opportunities for medical application. Oxidized cellulose is a very interesting material for biomedical research, due to its degradation in human body, hemostatic and antibacterial properties [6]. Collagen-based hemostatic agents have relatively low hemostatic activity in a wet environment, in systemic coagulopathies and thromboblastemia, infection risk. Collagen tends to lose the hemostatic capacity after autoclaving, which limits the application [7]. Oxidized cellulose is widely used in surgery for the treatment of skin lesions, long-term chronic wounds, liver, kidney resection, etc. Oxidized cellulose is insoluble in water, has a fibrous structure and high mechanical strength [8-10].

Therefore, the objective of the clinical study was to evaluate the effectiveness of Geprocel, a new domestic implant, in the treatment and prevention of disorders of aero- and hemostasis failures at lung surgeries.

MATERIAL AND METHODS

Hemostatic implant in the form of a fine powder was developed at RSRCS named after acad. V. Vakhidov". Geprotsel consists of the following components: the sodium salt of carboxymethyl cellulose, oxidized cellulose and nanocellulose associated with calcium ions (Patent No. IAP 20160273), in accordance with requirements of ISO 10993-1-2011.

A total of 69 patients operated at the department of lungs and mediastinum surgery of the Republican Specialized Research Centre of Surgery between 20015 and June, 2018 were included into this part of investigation.

All those patients had the risk development connected with air- and hemostasis both in intra-operative and in the postoperative periods. There were 35 patients in the main group (2017-2018) after resection phase or the lung parenchyma injury at the discharge from commissures. "Geprotsel" film has been applied over the defect of lung tissue for providing air- and hemostasis. 34 patients (2015-2017) were included into the group of comparison (comparable by age, sex, pathology, type of surgery and other objective criteria of contrastive analysis homogeneity).

Surgical procedure

The upper-midline laparotomy was performed under inhalation anesthesia (5% isoflurane). During the surgery, anesthesia was maintained by inhalation of 2- 2.5% isoflurane. The flat liver wounds of approximately 1 cm in diameter and 0.1 cm in depth were formed. Thus active parenchymal bleeding was stimulated (Figure 1). After suction, the hemostatic powder Heprocel was applied on the bleeding liver surface in Heprocel group. The control was treated only with standard gauze.

Ethical approval

The review board and ethics committee of RSCS named after acad. V.Vakhidov approved the study protocol and informed consents were taken from all the participants.

Statistical analysis

The obtained results were subjected to the statistical processing with the using the standard package of Microsoft Excel 2010 software by the method of variation statistics with the estimation of indexes' values (M±m).

RESULTS

The groups for comparison were representative by all main indices. In all cases during the intervention we noted the occurrence of injured part of pulmonary tissue parenchyma the form of which is depended on the resection type (organ lobe or its part) and also on the injury level during the discharge from commissures (echinococcectomy, decortications). Hemostasis in the area of pulmonary tissue injury (after acute resection at lobectomy or hardware wedge-shaped resection) was primary estimated after performing the main stage of surgery. In the comparison group we used standard methods for hemostasis achievement (tamponade, diathermo-coagulation, thermal effect). At the absence of the effect we conducted a sewing of bleeding area. In the main group "Geprotsel" film was initially

used for this aim – it was glue on the injured area with a fixation and a combined estimation of air- and hemostasis efficiency. Then we estimated hermeticity by conducting the test for airstasis. In the comparison group at the occurrence of air intake from organ parenchyma we also performed a fixation by additional sewing.

After performing the main stage of the surgery in 13 (37.1%) cases of the main group and in 12 (35,3%) cases of the comparison group there was noted non- intensive capillary bleeding from the injured part of pulmonary tissue. In the comparison group after using standard hemostatic procedures and estimation of airstasis efficiency in 13 (38.2%) cases we conducted the sewing of parenchyma defect and in 6 (17.6%) and in 4 (11.8%) patients the problem with air- and hemostasis was kept respectively. That is why they were undergone a recurrent sewing (10 – 29.4% cases). Hemorrhagic discharge through the drainage was determined in 3(8,8%) patients in the postoperative period and they were required additional hemostatic procedures. In other 2 (5.9%) patients we observed airstasis failure after surgery. We achieved positive clinical effect in the problem cases with both hemostasis and airstasis, but it influenced on the duration of pleural cavity drainage and then in 2 (5.9%) cases led to the development of the acute pleural empyema (Table 1).

Table 1. The frequency of intraoperative hemostasis and airstasis failures after anatomical or atypical resection of lung and additional sewing

Index	Main group		Comparison group	
	abs.	%	abs.	%
Intra-operative failures after resection	18	51.4%	17	50.0%
Hemostasis failure	13	37.1%	12	35.3%
Airstasis failure	5	14.3%	5	14.7%
Additional sutures on the lung tissue	4	11.4%	13	38.2%
Hemostasis failure	1	2.9%	6	17.6%
Airstasis failure	1	2.9%	4	11.8%
Total	2	5.7%	10	29.4%
Recurrent sewing of the lung tissue	2	5.7%	10	29.4%
Hemostasis failure (after surgery)	0	0.0%	3	8.8%
Airstasis failure (after surgery)	0	0.0%	2	5.9%
Total	0	0.0%	5	14.7%

Additional sutures on the lung tissue were required only in 4 (11.4%) cases in the main group after which they were kept only in 2 patients and then they were eliminated by recurrent fixation of sutures. There were no such complications in the postoperative period. It should be mentioned that after using the "Geprotsel" film we achieved an absolute air-and hemostasis in majority of cases and only in 4 (11.4%) patients we performed a recurrent sewing of the area with bleeding or affected airstasis. The problem with hemostasis or airstasis with the help of proposed biologic method, by our view, was connected with uneven surface of the injured area after lobectomy (2 cases), hardware sewing for wedge-shaped resection of peripheral benign tumor (neurofibroma - 1 case) and decortications (1 case). The applied film in those cases was not able to provide a complete hermiticity due to the tuberous surface of the defect – this area was additionally sewed and absolute hemostasis was achieved. The positive side of those cases is the fact that a biological material used for producing the "Geprotsel" film was used for getting another form of the implant – in the form of powder with analogous high adhesive properties providing an effective air- and hemostasis at application on small (up to 2-3 cm) uneven defect of pulmonary tissue parenchyma.

After singular application of fixing sutures on the pulmonary tissue the problems with airstasis were kept in 4 (11.8%) patients of the comparison group and only in1 (2.9%) patient of the main group. The problems with hemostasis were kept in 17.6% (6) and 2.9% (1) cases respectively (criterion χ^2=8.522; Df=3; P=0.047). In spite of the fact that additional fixing sutures had solved those problems, we noted airstasis failure in 2 (5.9%) cases and hemostasis failure in 3 (8.8%) patients of the comparison group in the postoperative period and in the whole it led to the development of these complications in 5 (14.7%) cases. The use of the "Geprotsel" film allowed to level completely the development risk of these complications in the postoperative period (criterion χ^2=9.107; Df=3; P=0.036). Subject to all intra- and postoperative failures of air- and hemostasis we reduced these complications indices in the main group from 44.1% (15 – comparison group; hemostasis - 9 (26.5%); airstasis - 6 (17.6%)) to 5.7% (2 – main group; 1 (2.9%) air-and hemostasis failures) (criterion χ^2=14.727; Df=3; P=0.003).

The necessity of achieving absolute air-and hemostasis was effected on the both duration of this stage and of surgery in the whole. The use of the "Geprotsel" film after surgery's main stage for leveling the complications development allowed to reduce the period for achieving air- and hemostasis from 32.8±2.5 minutes in the comparison group up to 12.5±1.2 minutes in the main group (T-criterion – 7.32; P<0.001). General duration of the surgery was reduced from 135.6±6.1 minutes т (comparison group) up to 107.2±4.7 minutes (T-criterion – 3.69; P<0.001) (Figure 1).

The complications development connected with air- and hemostasis failure in the postoperative period influenced on the duration of pleural cavity drainage. After 2-3 days the drain was removed in 97.1% (34 patients) in the main group and in 88.2% (31 patients) in the comparison group. After 4 days in 1 patient with airstasis failure of the main group the drain was also removed after its relief.

In 4 (11.8%) patients of the comparison group it was required a long term drainage with the drain removal after 5 days in 1 (2.9%) case, after 6-10 days in 2 (5.9%) cases and in 1 (2,9%) case the patient was discharged due to the development of acute empyema with further removal of the drain only at the achieving the complication regress after 33 days (Table 2).

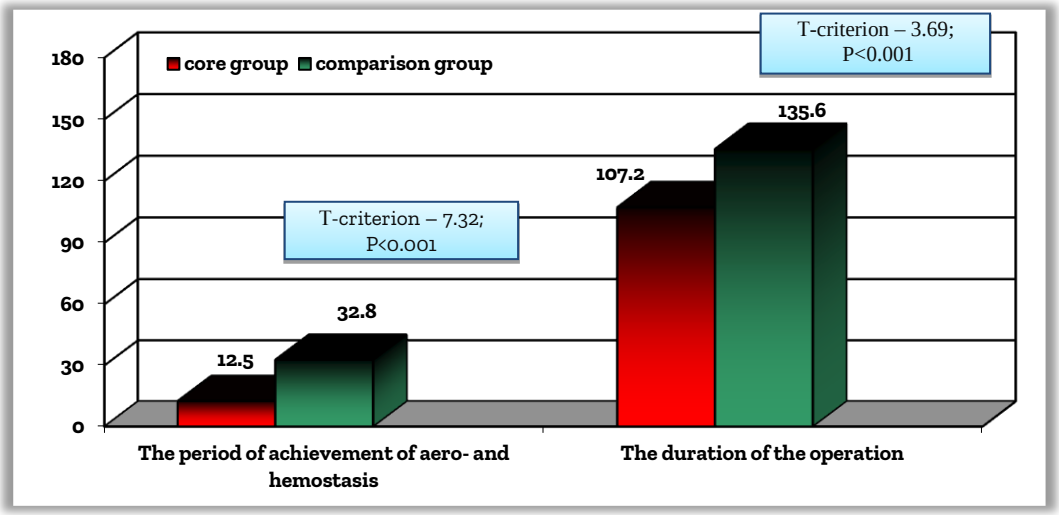

Figure 1. Average duration (minutes) of air- and hemostasis achieving period and the whole operative intervention

Table 2. The period of drain removal

Complication	Main group		Comparison group		Total	
	abs.	%	abs.	%	abs.	%
After 2 days	33	94.3%	29	85.3%	62	89.9%
After 3 days	1	2.9%	1	2.9%	2	2.9%
After 4-5 days	1	2.9%	1	2.9%	2	2.9%
After 6-10 days	0	0.0%	2	5.9%	2	2.9%
Discharged with drain	0	0.0%	1	2.9%	1	1.4%
Total	35	100%	34	100%	69	100%

At the mean data comparison of the pleural cavity drainage duration we noted a significant reduce of this index from 3.38±0.31 days in the comparison group up to 2.09±0.06 days in the main group (T-criterion – 4.09; P<0.001). The duration of the postoperative period on the background of intra-operative use of biological implant for air- and hemostasis reduced from 9.8±0.4 days up to 8.2±0.2 days (T-criterion – 3.58; P<0.01). The whole hospital stay was also significantly reduced from 12.1±0.4 days up to 10.7±0.2 days (T-criterion – 3.13; P<0.01). Summarizing the course of the postoperative period the following can be mentioned: intra-operative use of the domestic biological implant at the lung surgeries allowed to completely level the risk of air- and hemostasis failures in the postoperative period. In 3 (8.8%) cases of the comparison group we registered hemostasis failure and 2 (5.9%) cases of airstasis failure. The necessity in the additional fixing of sutures lines or defect zone of lung parenchyma after lobectomy in 1 case and in 1 case of decortications led to the significant

deformation of adjacent organ tissue and in its turn it led to the development of the syndrome of the lung low volume - 5.9% (Table 3).

This complication was noted only in 1 (2.9%) case of the main group. In other 2 (5.9%) cases of the comparison group on the background of long term drainage with airstasis failure and in 1 patient with low volume of the lung the acute pleural empyema was developed which was solved conservatively. The general frequency of complications in both groups reduced from 14.7% (5 patients in the comparison group) up to 2.9% (1 patient in the main group) (significance of differences by χ^2: 8.737; Df=5; P=0.043). There rate of complications frequency affected on the hospital stay duration. In proper time, 7-9 days after the surgery 88.6% (31 patients) of the main group and 67.6% (23 patients) of the comparison group were discharged. A prolonged hospital stay was required to 4 (11.4%) and 11 (32.4%) patients respectively (Table 4). Hereby, the implementation of domestic biological implant into the clinical practice at performing lung surgeries allowed to completely level the risk of postoperative failures of air- and hemostasis development, to reduce the general frequency of complications from 14.7% up to 2.9% (χ^2= 8.737; P=0.043) and the necessity of prolonged hospital stay from 32.4% up to 11.4%.

Table 3. Complications frequency in the postoperative period

Complication	Main group		Comparison group	
	abs.	%	abs.	%
Hemostasis failure	0	0.0%	3	8.8%
Airstasis failure	0	0.0%	2	5.9%
Low volume of the lung	1	2.9%	2	5.9%
Acute pleural empyema	0	0.0%	2	5.9%
Total	1	2.9%	5	14.7%
Significance of differences (χ^2 criterion)	8.737; Df=5; P=0.043			

Table 6. The frequency of prolonged hospital stays in the postoperative period

Complication	Main group		Comparison group	
	abs.	%	abs.	%
Discharged in standard period (after 7-9 days)	31	88.6%	23	67.6%
Prolonged hospital stay	4	11.4%	11	32.4%
Total	35	100%	34	100%

DISCUSSION

In surgery, the prevention of postoperative complications has always been and remains relevant. In thoracic operations, it is known that the leading determining factor in the prevention of respiratory disorders and the prevention of infectious complications is the fastest and most complete smoothing of the lung in the postoperative period. One of the most important components contributing to this, in addition to effective drainage, restoration of muscle tone and adequate breathing, is reliable aerostasis and hemostasis.

In a study by Wain et al. [11] showed that a violation of the tightness of the lung suture intraoperatively occurs in 70% of cases. According to the European Society of Thoracic Surgeons (ESTS), the incidence of long-term aerostasis failure after marginal resection of the lung and lobectomy is 3.5% and 8.3%, respectively [12]. Long-term failure of aerostasis is always associated with the need for prolonged drainage of the pleural cavity, an increase in the duration of inpatient treatment, and an increased risk of developing infectious complications. The European Society of Thoracic Surgeons defines the failure of aerostasis as prolonged with air discharge for 5 days or more after surgery. Brunelli et al. [13, 14] in studies on the risk factors for leakage of the seam of the lung, they also determine long-term failure of aerostasis for a period of 7 days or more.

Modern approaches in the prevention of postoperative complications associated with lack of aero- and hemostasis in the literature are based on the use of new technologies to strengthen the bronchial suture. Nevertheless, the literature data on the effectiveness of the use of various patches are contradictory in many respects. Along with the use of traditional materials, there is an active search and development of materials based on bio-base.

The most promising means of biological hemostasis are fibrin polymers. Their main advantage is that they completely consist of biological blood components and, when applied to the damaged area, imitate the

physiological mechanism of hemostasis. However, fibrin compositions are usually two-component and are applied to tissues with the help of injection needles, nebulizers, catheters. Moreover, two-spray applicators are used, which creates certain difficulties in their use in thoracic surgery.

Long-term use of cellulose in the form of a dressing material is experiencing a new period of using its derivatives, which, depending on the type and degree of polymerization, can be widely used in surgery as an independent active principle as a bioinert non-toxic biodegradable implant with certain physical and chemical properties as well as medical properties.

CONCLUSION

The issues of prevention and treatment of air- and hemostasis failure still remain actual in the modern lung surgery. It is especially actual for those patients who have chronic obstructive lung disease, emphysematous injuries and other concomitant diseases of respiratory system. During the lung surgeries in patients with chronic obstructive lung disease, emphysema the risk of these complications development can reach up to 11.8% after lobectomy, after wedge-shaped resections – up to 9.1% and after decortications – up to 33.3%. In whole by all surgeries it makes up 14.7% (airstasis failure – 5.9% and hemostasis failure - 8,8%). The use of the "Geprotsel" biological implant allowed to reduce a necessity of the additional single sewing of the pulmonary tissue for providing air- and hemostasis from 38.2% up to 11.4% and multiple fixing by sutures from 29.4% up to 5.7% (χ^2= 7.706; Df=2; P=0.021).

DECLARATIONS

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Authors' contributions

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests.

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Current status of stem cell therapy

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ABSTRACT

Introduction. Stem cells have the extraordinary potential to develop into many diverse cell types in the body during early life and growth. Significant progress has been made in understanding the biochemical and metabolic mechanisms and feedback associated with different stem cells response. Some of the challenges concerning transplanted embryonic stem cells and mesenchymal stem cells are immune-mediated rejection, senescence-induced genetic instability or loss of function, and limited cell survival. Aim. The aim of this review, is to recapitulate the recent status and information about the use of embryonic stem cells and mesenchymal stem cells for research into how cells and tissues of the body grow and develop, and potentially useful for curing disease. Results. Stem cell therapy efforts are currently underway for virtually every type of tissue and organ within the human body. Because the current status of stem cell incorporates the fields of cell transplantation, materials science, and engineering, personnel who have mastered the techniques of cell harvest, culture, expansion, transplantation, and polymer design are essential for the successful application of this technology. Various stem cell therapies are at different stages of development, with some already being used clinically, a few in preclinical trials, and some in the discovery stage. Recommendations. Recent progresses suggest that stem cell therapy may have expanded clinical applicability in the future because they represent a viable therapeutic option for those who require tissue and cells replacement in diverse degenerative disease. More recently, major advances in the areas of stem cell biology, tissue engineering, and nuclear transfer techniques have made it possible to combine these technologies to create the comprehensive scientific field of regenerative medicine. "But there is a strong need for better understanding the biology, manipulation and safety of stem cells in tissue regeneration and repair before starting the therapeutic applications."

Review

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INTRODUCTION

Modern treatments for numerous degenerative diseases like Alzheimer disease, Parkinson disease, motor neuron disease, multiple sclerosis, diabetes, and kidney, liver, and heart diseases, as well as for several types of cancer, are mostly symptomatic, and for certain diseases, total recovery implies entire organ transplantation [1, 2]. Numerous applications of stem cells in tried and validated therapies are recognized in humans: starting from bone marrow transplants to more recent advances in skin and cornea repair [3]. Stem cell transplantation would probably have to be achieved within the window of time between the first appearance of injury and irreparable loss of neurons [4].

Up to date advancement shows that stem cell therapy that concerns cell reprogramming and transplantation of Embryonic Stem Cells (ESCs), Mesenchymal Stem Cells (MSCs) and induced pluripotent stem cells (iPSCs) represents an interesting so far disputed research area, with exciting results for many diseases [3, 5, 6]. Human iPS cell derivation previously required vectors that integrate into the genome, which can create mutations and limit the utility of the cells in both research and clinical applications [7].

The use of stem cells in the clinical field has gathered unbelievable momentum over the last decade, advanced by varying levels of achievement in clinical trials and by the advancement in our understanding of the mechanisms by which stem cells exert their seemingly favorable effects. Generally speaking, stem cells can be characterized as either embryonic or adult stem cells [8]. Stem and progenitor cells from adult tissues represent an important promise in the therapy of a number of pathological conditions [9].

Stem cell transplantation is being widely investigated as a potential therapy for cell death-related heart diseases [10]. This rapid translation into clinical studies has left a lot of questions concerning cell therapy unanswered [11, 12]. There is rising evidence that stem cells secrete a variety of growth factors, cytokines,

chemokines and bioactive lipids that control their biology in an autocrine or paracrine-manner and orchestrate interactions with the surrounding microenvironment [13].

The 21st century is witnessing an uprising in cellular therapy. Stem cell technology is proving to be a valuable tool not only for the development and regeneration of various tissue and organ systems, but also as a unit in evolution by natural selection [14]. Recently stem cell therapies are assumed to be used as safe and effective treatments. Even applications of stem cells are being investigated in clinical trials, including the use of stem cells to regenerate damaged tissues such as heart, skin, bone, spinal cord, liver, pancreas and cornea or to treat blood or solid-organ cancers [15].

So that stem cell research is a new field that is advancing at a hard to believe pace with new discoveries being reported from all over the world. Scientists have for years looked for ways to use stem cells to replace cells and tissues that are damaged or diseased. The miracles of stem cell application in incurable clinical conditions are being reported through media and newspapers [16]. To date, stem cell types which have been used in clinical trials include hematopoietic stem cells (HSCs), mesenchymal stem cells, neural stem cells, epidermal stem cells, endothelial progenitor cells, limbal stem cells, embryonic stem cells, and induced pluripotent stem cells [17]. The main properties that characterize stem cells include their indefinite capacity to renew themselves and leave their initial undifferentiated state to become cells of several lineages [18].

Heart failure (HF) is a leading cause of disability and death that accounts for approximately one million hospitalizations, over 50,000 deaths, and almost $35 billion in health care costs in the United States each year [19]. The use of stem cells in cardiology is frequently characterized as a matter of providing new myocytes, but it is much more complex than that. Whether global or segmental, heart failure is generally due to a specific cause, which must be removed as a precondition for the success of any reconstructive effort. Likewise, the mere generation of new vessels (by means of angiogenesis or vasculogenesis) [20]. Even more important, unlike the progenitor cells used in bone marrow transplants, the elementary myocardial functional units are not lone cardiomyocytes but, rather, are myocardial cells that are integrated into a multicellular assembly of myofibers. These cells are oriented in specific directions (indeed, implanted cell therapy should avoid generating myofiber disarray, which is a disease state in itself). Therefore, the challenges of stem cell treatment for the heart are much more complex than those of blood transfusion for anemia and bone marrow transplantation for bone marrow failure, which is the only clinically successful cellular treatments thus far [19].

Heart transplantation remains the ultimate approach to treating heart failure, but this is costly and excludes patients who are poor candidates for transplantation given their co-morbidities, or for whom a donor organ is unavailable. Stem cell therapy represents the first realistic strategy for reversing the effects of what has until now been considered terminal heart damage [21]. Therefore, in this review, We attempted to summarize the current status, available evidence, and present several clinical and nonclinical data concerning mainly the use of ESCs and MSCs in the treatment of different cardiovascular disease, highlighting both the opportunities and the limitations of stem cell therapy.

CURRENT STATUS OF STEM CELL

Embryonic stem cell

Since human embryonic stem cell (HESC) lines were first derived in 1998, these cells have been in high demand as objects of research. The ability of HESCs to reproduce almost limitlessly and to differentiate into many, if not all, cell types of the human body have generated an enormous amount of scientific interest. These unique capabilities provide a means of exploring many promising lines of research, which are likely to reveal a deeper understanding of human cellular biology and which may lead to potential cures for many diseases [22]. Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation in vitro. The term "ES cell" was introduced to distinguish these embryo-derived pluripotent cells from terato-carcinoma-derived pluripotent embryonal carcinoma (EC) cells [6].

Derivation of human embryonic stem cell (HESC)

HESC lines are conventionally derived from the inner cell mass (ICM) of pre-implantation stage blastocysts, of both good and poor quality, which have been donated for research and would otherwise be discarded. Morula-stage embryos or late-stage blastocysts (7-8 days) may also be used to create HESC lines. Although all the HESC lines derived worldwide share the expression of characteristic pluripotency markers [23]. Many differences are emerging between lines that may be more associated with the wide range of culture

conditions in current use than with the inherent genetic variations of the embryos from which HESC were derived [24].

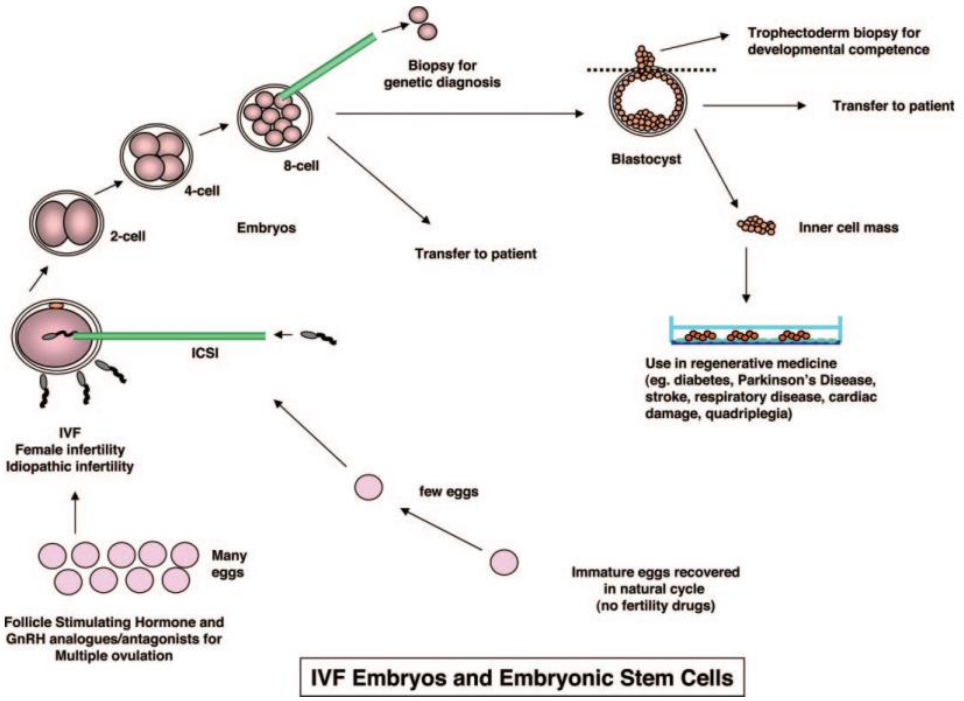

Figure 1. Derivation of HESC (human embryonic stem cell) [25].

Colonies of HESCs differ from the ICM in a number of ways. Firstly, ICM cells retain a memory for axes, dorsal-ventral, anterior-posterior, and left-right axes, that enables the differentiating cells to have position relationships that guide the differentiation, expansion, and integration of cell types required to form an organism. It is generally considered that ESCs are an epiblast derivative, or even a type of germ stem cell, that can be maintained as an immortal and pluripotential cell type under strict laboratory conditions, in the presence of secretory products of embryonic, or adult, somatic cells. Importantly, the self-renewal of HESCs appears to involve the Wnt family signaling pathway and probably other pathways that involve basic fibroblast growth factor (bFGF) and TGF-β [23].

In 1998, Thomson *et al.* [6] were as a first reporter of the successful derivation of HESCs from preimplantation human embryos. Their report followed they extensive studies by Thomson *et al.* [26] on the production of rhesus and marmoset ESCs. Intact blastocysts and mechanically isolated ICMs grown on mouse embryonic fibroblasts (STO cells) they are studied by the research group in Singapore from 1994–1996, and these cultures resulted in cell lines that differentiated after several passages *in vitro* [27].

The methods finally used successfully to establish HESC lines were described by Reubinoff *et al.* [28]. These methods were similar to those described by Thomson *et al.* [6, 29] and involved the isolation of ICM clusters from human blastocysts by immunosurgery and their co-culture with mitotically inactivated murine embryonic fibroblasts (MEFs). The HESCs form typical colonies of undifferentiated cells that need to be passaged weekly likely or, more often, as mechanically dissected colonies of 10 cells or more. Additional HESC lines have been derived by similar methods. More recently HESCs have also been derived under feeder-free conditions using cell-free lysates of MEFs [30].

The selection criteria used for choosing human embryos for deriving HESCs will determine the eventual success rates for their production. Small numbers of blastocyst-stage embryos grown in co-culture with human oviductal epithelial cells they are used by Reubinoff *et al.* [28] to produce six HESC lines after preliminary experiments involving around 30 embryos [23]. The six HESC lines they are derived from 12 blastocysts. This very high success rate of producing HESCs can be compared with the use of much larger numbers of embryos (blastocysts) by others. It is probable that about 50% of human embryos have chromosomal abnormalities, and it would be expected that these genetic errors would limit the success rate of HESC production. It is also difficult to establish HESCs from monosomic or trisomic embryos, with less than 10% made from human

embryos diagnosed as aneuploid. Interestingly, two HESC lines produced from trisomic embryos reverted to diploidy, indicating the embryos they are probably mosaic [31].

A large number of HESC lines have been produced from excess human IVF embryos by some IVF clinics; for example, Kukharenko *et al.* [32] reported 46 new HESC lines made from morulae, blastocysts, and ICMs isolated from blastocysts [33]. There was apparently little difference between stages of preimplantation human embryos in their capacity to form HESC lines. A more recent comparison of mechanical isolation of ICMs and plating whole blastocysts for deriving new HESC lines showed that mechanical isolation is more efficient. The use of antiserum raised in animals for immunosurgery to isolate ICMs is undesirable [34].

Genetic manipulation of human embryonic stem cell (HESCs)

Clonal derivation of HESCs is difficult, and the efficiency is extremely low [35]. However, it is possible to transfect HESCs with DNA constructs, and this is important for determining the role of transcription factors for the renewal and differentiation of HESCs. Identification of specific gene expression by reporter genes enables purification of cells of interest in differentiating cultures and the tracking of HESC derivatives in mixed cell cultures or when transplanted into animal models. Conventional transfection methods have been successful [36], as have lentiviral methods. Integration of reporter genes into controlling elements of specific genes or the approach of gene knock out or knock in used for functional genomics is very difficult because of the inability to clone HESCs. However, Zwaka and Thomson [37] have shown that it is possible to electroporate HESCs to achieve homologous recombination of HESC colony fragments. Gene function may be more appropriately determined in HESCs by using small inhibitory RNAs [38] to control renewal, differentiation, apoptosis and other mechanisms involved in cell function and response to internal and external stimuli.

Markers of human embryonic stem cell (HESCs)

Sperger *et al.* [39] have reported that, by microarray analysis, 330 genes are highly expressed in common in HESCs and human embryonal carcinoma cells and seminomas. This included *POU5F1* (*Oct4*) and *FLJ10713*, a homolog highly expressed in mESCs. Among those genes only highly expressed in HESCs and human embryonal carcinoma cells included a DNA methylase (*DNMT3B*), which functions in early embryogenesis, and *Foxd3*, a fork head family transcription factor that interacts with *Oct4*, which is essential for the maintenance of mouse primitive ectoderm [40]. *Sox2* is also highly expressed and is known to be important in pluripotentiality for example: The derivation of neural progenitor cells from human embryonic stem (ES) cells is of value both in the study of early human neurogenesis and in the creation of an unlimited source of donor cells for neural transplantation therapy. Here we report the generation of enriched and expandable preparations of proliferating neural progenitors from human ES cells. The neural progenitors could differentiate in vitro into the three neural lineages-astrocytes, oligodendrocytes, and mature neurons. When human neural progenitors were transplanted into the ventricles of newborn mouse brains, they incorporated in large numbers into the host brain parenchyma, demonstrated widespread distribution, and differentiated into progeny of the three neural lineages [41]. Embryonic stem (ES) cells are cells derived from the early embryo that can be propagated indefinitely in the primitive undifferentiated state while remaining pluripotent; they share these properties with embryonic germ (EG) cells. Serial analysis of gene expression (SAGE) has been reported by Richards *et al.* [42] and has been compare with some cancer SAGE libraries. As expected, *Oct4*, *Nanog*, and *Sox2* transcripts appear abundantly, but there were differences between HESCs in some other transcript abundance (*e.g.*, *Rex-1*).

Patient-Specific Stem Cells

There is much interest in the production of patient-specific stem cells using nuclear transfer techniques to introduce somatic cell nuclei into enucleated oocytes [23]. The reason for making HESCs for individual patients is for the possible establishment of immune-compatible cell derivatives for transplantation. It is important that new disease-specific stem cells be derived from patients with cancers; neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, motor neuron disease, and multiple sclerosis; and others of unknown cause or multigenic origins. The ability to reestablish pristine HESCs that can be differentiated in the laboratory to cells that will express the disease phenotype could be a very valuable resource for screening for molecules that interfere with the disease phenotype and identifying candidate drugs or molecular pathways that may enable a whole new approach to pharmaceuticals for these patients. This approach has already proven productive using mESCs [43].

Mesenchymal Stem Cells (MSCs)

Several progenitor cells can be found in human adult bone marrow. One class of multipotent adult progenitors is referred to as mesenchymal stem cells (MSCs). It is well documented that these cells are capable of differentiating into bone, cartilage, muscle, marrow stroma, tendon and ligament, fat, and a variety of other connective tissue [44]. Like the hematopoietic stem cells (HSCs) of marrow, the differentiation of MSCs involves multi-step cell lineages controlled by bioactive factors found in the local micro-environment or supplied in the culture environment of *ex vivo* cultivated cells. This controlled differentiation scheme was evolutionarily selected because it comprises a sequential process that can be modulated both in time and end-stage outcome; a multi-step pathway allows a large number of regulatory elements to be used to safeguard the final outcome [45]. Mesenchymal stem cells (MSCs), also referred to as connective tissue progenitor cells or multipotent mesenchymal stromal cells, have demonstrated significant potential for clinical use. Thus, MSCs have been the focus of a regime of emerging therapeutics to regenerate damaged tissue and treat inflammation resulting from cardiovascular disease and myocardial infarction (MI), brain and spinal cord injury, cartilage and bone injury, Crohn's disease, and graft-versus-host disease (GVHD) during bone marrow transplantation [46].

As part of the minimal criteria, human MSCs must adhere to tissue culture plastic; be positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14 or CD11b, CD79a, or CD19 and HLA-DR; and must be able to differentiate to osteoblasts, adipocytes, and chondroblasts under standard *in vitro* differentiating conditions [47].

Tissue sources of Mesenchymal Stem Cells (MSC)

The reported MSC frequency (as measured by CFU-F) and native concentration from several adult human tissues are reported. The relative abundance of MSCs throughout the body is understandable in light of recent findings that most, if not all, MSCs are of perivascular origin. Furthermore, there is a direct correlation between MSC frequency and blood vessel density in stromal vascularized tissue [48]. MSCs and pericytes share the phenotypic surface markers melanoma cell adhesion molecule (CD146) and platelet-derived growth factor receptor. It is hypothesized that pericytes are the in *vivo* source of MSCs, with cellular components protruding into the endothelial lumen of blood vessels to monitor and react to systemic signals. The widespread distribution of perivascular precursors for MSCs would account for their ability to respond to injury by sensing and secreting chemokines locally in response to injury, infection or disease in all vascularized tissues of the body [49].

Capacity of Mesenchymal Stem Cells (MSC)

Trophic properties of MSC: The primary trophic property of MSCs is the secretion of growth factors and other chemokines to induce cell proliferation and angiogenesis. MSCs express mitogenic proteins such as transforming growth factor-alpha (TGF-α), TGF-β, hepatocyte growth factor (HGF), epithelial growth factor (EGF), basic fibroblast growth factor (FGF-2) and insulin-like growth factor-1 (IGF-1) to increase fibroblast, epithelial and endothelial cell division. Vascular endothelial growth factor (VEGF), IGF-1, EGF, and angiopoietin-1 are released to recruit endothelial lineage cells and initiate vascularization [50].

Anti-inflammatory and immunomodulatory properties of MSC: MSCs hold up via paracrine mechanisms and change the regenerative environment via anti-inflammatory and immunomodulatory mechanisms. In response to inflammatory molecules such as interleukin-1 (IL-1), IL-2, IL-12, tumor necrosis factor-a (TNF-a) and interferon-gamma (INF-g), MSCs secrete an array of growth factors and anti-inflammatory proteins with complex feedback mechanisms among the many types of immune cells [49]. The key immunomodulatory cytokines include prostaglandin 2, TGF-b1, HGF, SDF-1, nitrous oxide, indoleamine 2, 3-dioxygenase, IL-4, IL-6, IL-10, IL-1 receptor antagonist and soluble tumor necrosis factor-a receptor. MSCs prevent proliferation and function of many inflammatory immune cells, including T cells, natural killer cells, B cells, monocytes, macrophages and dendritic cells [51].

Anti-apoptotic properties of MSC: In a situation where MSCs are administered with the aim of treating acute lesions, the first expected effect is the reduction of the extent of cell death, and this is observed in animal models of tissue injury and in co-culture experiments. Togel *et al*. reported that infused MSCs attach to the renal micro-vascular circulation and decrease apoptosis of adjacent cells in a model of acute kidney injury. In

order to elucidate the factors responsible for the observed renoprotective effect, these authors analyzed the MSC-conditioned medium and verified the presence of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin-like growth factor 1 (IGF-1), factors that enhance endothelial cell growth and survival [48]. Parekkadan *et al.* [52] found the presence of these and other anti-apoptotic molecules in MSC-conditioned medium and, interestingly, showed that an MSC-containing bioreactor connected to the bloodstream of rats experimentally subjected to fulminant hepatic failure resulted in the survival of 71% of the animals in contrast to 14% survival in the control group.

MSCs reduce apoptosis of UV-irradiated fibroblasts and lung epithelial tumor cells cultured under low pH and hypoxia, and the up-regulation and secretion of stanniocalcin-1 has been found to be at least partially responsible for this anti-apoptotic effect [53]. Also, adipose tissue-derived MSCs have been shown to express HGF, VEGF, transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF, aka FGF2) and granulocyte–macrophage colony-stimulating factor (GM-CSF), and the expression of these molecules was found to increase under hypoxic culture conditions; particularly, VEGF upregulation under hypoxia has been shown to be greater than that observed for other factors [54].

Hypoxia takes place in the first stages of tissue injury, and secretion of anti-apoptotic factors by MSCs at this stage minimizes the extent of cell death in the tissues surrounding the injured areas; accordingly, in the latter study, it was further demonstrated that cultured, adipose-derived MSCs reduce necrosis and improve perfusion when injected into mice experimentally subjected to hind limb ischemia. Scientist's suggest that this anti-apoptotic activity could serve to limit the field of injury *in vivo* circumstances [54].

Table 1. Anti-inflammatory mechanisms of MSCs

Target Cell	Mechanism	Primery Effect	Secondary Effect
Dendritic cells	PGE2/direct contact	↓TNF-đ IL-12. differentiation and activation	↓Impairs effect on resting NK cells
Immature Dendritic cells	PGE2, IL-6. IL-8 and SDF-1 PGE2	↑IL-10	↓T.ceII proliferation
T cells (CD4 +, helper T cells)		↑IL-10	↓INF--, byTH1 cells'
T cells (CD8 +, Cytotoxic T-cells) Treg cells	IL-10, sHLAG5,IL-10	↓CD4 + T-cell proliferation by	↓rIL-4 by 1H2 ceilsa
		↓S-phase entry block and ↓Go/G1 phase arrest	↓ Treg production.
B-Cells	IL.10		IL-10 by Treg cells
	sHLAG5	↓Inhibits T-ceII functions	↓B-cell proliferation
NK-Cells	IL-10	↓InactivateT_H1- cells	↓Ig antibody production
		↓Cytotoxicity	↓by B cells
Monocytes	sHLA-G5	↑Treg Proliferation	
	PGE2. TGF- β 1, TGF-1, IDO. NO and PD-L1	↑IL-10 by Treg cells	
Macrophages	PGE2. IDO. HLA.G5. HGF. TGF- β 1	↓Trq differentiation	↓TNF-X and IL-1
Neutrophils	PGE2		
	IL-6		

Abbreviations: HGF, hepatocyte growth factor; HLA, human leukocyte antigen; IDO, indoleamine 2,3-dioxygenase; IL-1Ra, IL-1 receptor antagonist; INF, interferon; MMP, matrix metalloproteinase; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cell; NK, natural killer; NO, nitrous oxide; PD-L1, programmed cell death ligand-1; PGE2, prostaglandin 2; SDF-1, stromal cell-derived factor-1; sTNF-R, soluble TNF-a receptor; TGF, transforming growth factor; TNF, tumor necrosis factor; TSG, tumor necrosis alpha-stimulating gene; VEGF, vascular endothelial growth factor. A Promotes TH1-TH2 T-cell transition [46].

Antimicrobial properties of MSC: Assessment of direct inhibition of bacterial growth by MSCs or its conditioned medium (CM) was done by counting CFU. In brief, MSCs in 24-theyll plates (2×10^5 cells per well) in RPMI supplemented with 5% FBS were infected with 300 CFU *E. coli* or *S. aureus* and incubated for 6 hours in humidified CO_2 incubator, then aliquots of culture medium were taken from each well, serially diluted with

sterile PBS, and plated on LB-agar plates (Teknova, Hollister, CA). Colonies were counted after overnight incubation at 37°C. Antimicrobial activity of MSC CM (or synthetic LL-37) was tested by a Microdilution susceptibility test according to Andra *et al.* [55].

The researcher that studied human MSCs might express direct antimicrobial activity through the secretion of antimicrobial peptides. They examined the effect of human MSCs on bacterial growth *in vitro*. Expression of different antimicrobial peptides was investigated using reverse transcription polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and immuno-histochemistry. Following stimulation with live *E. coli*, human MSCs produced one candidate antimicrobial peptide, LL-37, which was subsequently found to be responsible for antimicrobial activity in vitro. To determine if the secretion of LL-37 by MSCs would alter bacterial clearance in vivo, they tested BM-derived human MSCs in an *E. coli* pneumonia model in mice. Treatment with human MSCs, given 4 hours later, resulted in a significant reduction of *E.coli* colony-forming unit (CFU) in the lung homogenates (LHs) and the bronchoalveolar lavage (BAL) fluids. The effect was blocked with a neutralizing antibody to LL-37 demonstrating that human MSCs possessed antimicrobial activity, which is explained in part by the secretion of LL-37 [56].

Phenotypic characterization of Mesenchymal Stem Cells (MSC): After the discovery and early characterization of MSCs, scientists desired a method to prospectively isolate progenitor cells from bulk populations based upon positive or negative selection of CD markers expressed by the cells. The first markers unquestionably identified on MSCs were CD73 (SH-3/4) and CD105 (endoglin or SH-2), followed thereafter by CD90 (Thy- 1) and CD44. It since has been discovered that the quadruple-positive population of CD90þ/CD105þ/CD73þ/CD44 [57, 58]. It is common to fibroblasts and stromal cells, and only serves to discriminate these cell types from those of hematopoietic origin. Significant MSC phenotypic characterization has been published in the interim, but unfortunately there remains no strict consensus among the field [59]. In 2006, the International Society of Stem Cell Research established a minimum set of criteria for defining MSCs as: (1) plastic-adherent cells; (2) capable of tri-lineage (bone, cartilage and fat) differentiation; (3) phenotypically positive for CD105, CD73 and CD90; and (4) negative for CD45, CD34, CD11b, CD14, CD79a and HLA-DR [60]. However, these criteria are based on the characterization of *in vitro* cultured cells and do not apply to the native *in vivo* phenotype. For example, CD34 is considered a marker for hematopoietic stem cells and endothelial progenitors for freshly harvested cells in BM aspirate, but not MSCs [61].

Pericytes are stimulated by soluble growth factors and chemokines to become activated MSCs, which respond to the microenvironment by secreting trophic (mitogenic, angiogenic, anti-apoptotic or scar reduction), immunomodulatory or antimicrobial factors. After the microenvironment is re-established, MSCs return to their native pericyte state attached to blood vessels [55].

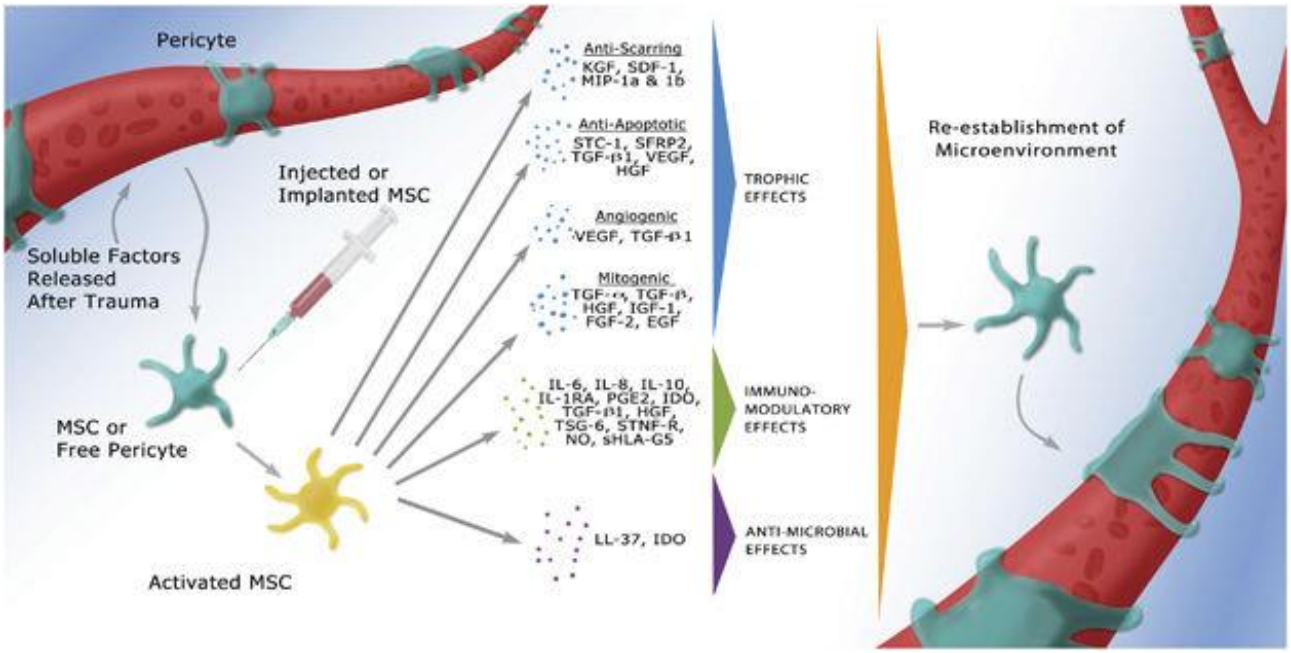

Figure 2. Phenotypic characterization of MSC.

CLINICAL TRANSLATION OF STEM CELL THERAPIES

Mesenchymal stem cells (MSC) in the treatment in cardiovascular therapies

Cardiac: Myocardial infarction is a multi-faceted insult to the cardiovascular system, stemming from the initial ischemic event; the extent of damage and subsequent cardiac disease correlates with the size of the original infarcted region [62, 63]. It is characterized by the disruption of blood supply to the heart muscle cells, which lead to myocardial infarction or death of cardiomyocytes. Reperfusion therapy or the restoration of blood flow by thrombolytic therapy, bypass surgery or percutaneous coronary intervention (PCI) is currently the mainstay of treatment for AMI and is responsible for the significant reduction in AMI mortalit. The efficacy of reperfusion therapy has led to increased survival of patients with severe AMI who would not otherwise survive. However, many (23%) of these survivors progress to fatal heart failure within 30 days. This phenomenon of an increasing number of severe AMI survivors contributes to an ever growing epidemic of heart failures [64].

Frantz *et al.* [63] have proposed the possibility of anti-inflammatory agents for minimization of deleterious post-myocardial infarction tissue remodeling. Several clinical studies have recently investigated the use of MSCs for this purpose; however, there has been no consensus yet on the preferred delivery method or type of cell. In a randomized, placebo-controlled study of chronic myocardial infarction patients receiving intra-myocardial injections of autologous BM-derived mononuclear cells, cell therapy patients had a decrease in summed stress score and increase in left-ventricular (LV) ejection fraction at 3 and 6 months (both statistically significant) [65, 66]. A subsequent study of 87 patients with severe LV dysfunction revealed no statistical differences in LV ejection fraction or size of infarct between placebo and autologous BMNC infusion [67]. A much smaller study revealed that both autologous BM MNCs and expanded BM MSCs yielded a decrease in myocardial scarring by 3 months, indicating beneficial tissue remodeling [68].

Similarly, the percutaneous stem cell injection delivery effects on neomyogenesis (POSEIDON) randomized trial comparing allogeneic and autologous MSCs in 30 ischemic cardiomyopathy patients indicated increased functional capacity, quality-of-life and ventricular remodeling as a result of both allogeneic and autologous cell therapy [69]. Most recently, direct myocardial injection of autologous, expanded BM MSCs resulted in persistent improvements in exercise capacity, Canadian cardiovascular scale (CCS) class score, angina attack frequency and nitroglycerin consumption at one-year post-intervention [70].

Opportunities and limitations of stem cell therapy

One of the limitations of applying cell-based regenerative medicine techniques toward organ replacement has been the inherent difficulty of growing specific cell types in large quantities [2]. Another obstacle that remains to be fully elucidated is the potential immune response to an ES and MSCs cell derived tissue graft and immune-mediated rejection, senescence-induced genetic instability or loss of function, and limited cell survival. This is demonstrated by the fact that nude mice, which lack T cells, are unable to mount a rejection response against an allogeneic skin graft. The unique ability of ES cells to give rise to HSC offers an interesting potential whereby immunological tolerance can be induced via hematopoietic chimerism [71].

Build out of regenerative service lines is predicated on effective clinical-grade biotherapies suitable for scale-up and standardized production and application. A viable supply chain requires quality-controlled manufacturing and delivery of products that fulfill patient specifications. Patient modifiers such as age, sex, morbidities, and concomitant therapies impact regenerative fitness. Cell performance is also subject to influences during procurement, production, and/or delivery. In fact, not all individuals harbor stem cells with a uniform reparative capacity [72].

CLINICAFUTURE PERSPECTIVES

The past decade has improved our knowledge of stem cell biology and the development of the cardiovascular system. However, a more profound understanding of cardiac myogenesis will be required for the development of advanced stem cell therapeutics to repair or regenerate damaged money disease [73]. The future will likely include (i) further investigation to delineate the human CM lineage tree; (ii) methods to isolate specifc cardiac progenitor pools or specialized CM subtypes; (iii) strategies to ensure survival of transplanted cells, their functional integration with the host myocardium, and circumvention of immune rejection; (iv) development of technologies to accurately assess integration; (v) determination of parameters that optimize engraftment, such

as delivery method, timing of transplantation post-MI, and cell preparations; and (vi) large-animal models of heart failure that closely resemble human cardiovascular physiology and disease for assessing cell engraftment, host immune response, and myocardial function [74].

Cell-replacement therapies hold great potential for treating Alzheimer's disease and related disorders patients. With the advent of stem cell technologies and the ability to turn stem cells into different types of CNS neurons and glial cells, some success in stem cell therapy has been made in animal models of Alzheimer's disease. Although these preclinical studies are promising, many more steps remain before stem cell therapies can be successfully used for the treatment of Alzheimer's disease and related disorders [75].

CONCLUSION

Stem cells therapy is under investigation for a number of therapeutic applications. These cells are known to home to some tissues, particularly when injured or under pathological conditions. The mechanisms underlying migration of MSCs and ESCs remain to be clarified, although evidence suggests that both chemokines and their receptors and adhesion molecules are involved. Different studies describe the role of chemokine receptors and adhesion molecules on stem cells may allow the development of therapeutic strategies to enhance the recruitment of *ex vivo*-cultured MSCs to damaged or diseased tissues. This could lead to various therapeutic possibilities such as supporting tissue regeneration, correcting inherited disorders (e.g., of bone), dampening chronic inflammation, and using these cells as vehicles for the delivery of biological agents. Further clinical data are necessary, however, to determine the *in vivo* distribution and therapeutic mechanisms of MSCs and ESCs to optimize their use as part of a personalized regenerative medicine strategy. This process will require the collaborative efforts of physicians, veterinarian, scientists, biotechnologiests, industry and regulatory agencies to translate nature's basic regenerative element into the continuum of clinical care. Stem cells are the potiential area for research and doing new regenerative engnering and cell therapy at the cell levels.

DECLARATIONS

Authors' contributions

MB conceived the review, coordinated the overall activity, and reviewed the manuscript. AK and MY supervising all in all activities.

Availability of data and materials

Data will be made available upon request of the primary author.

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Competing interests

The authors declare that they have no competing interests.

Consent to publish

Not applicable.

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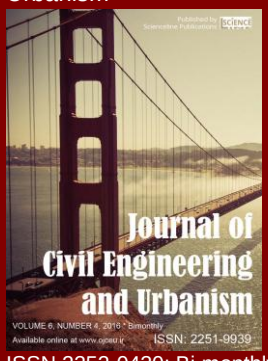

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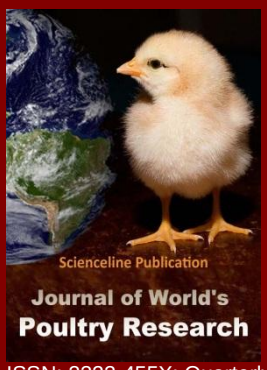

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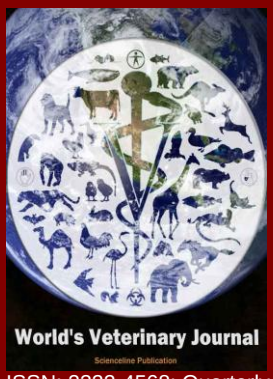

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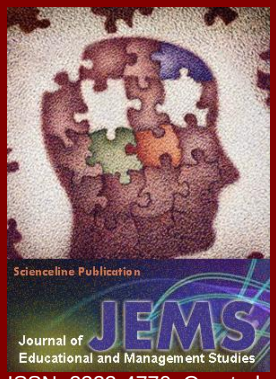

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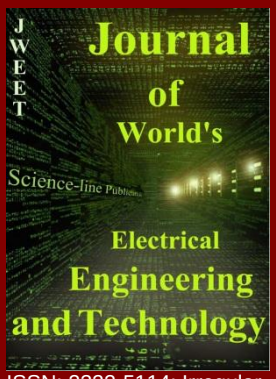

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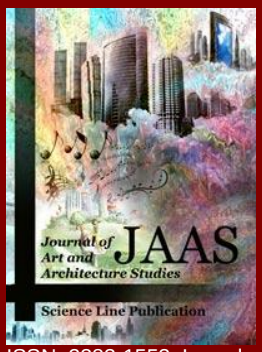

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