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Original Article

The effect of umbilical cord blood stem cells and micro connection bridge system on rat spinal cord injury

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Abstract: Objective: To investigate the effect of umbilical cord blood stem cells (UCBSC) and mechanical micro connection system (mMS) on new vessel formation and cyst after spinal cord injury in rat, and analyze the correlation with Basso Beattie Bresnahan (BBB) score. Methods: All the rats were randomly divided into four groups: medium group, UCBSC group, mMS group and mMS & UCBSC group. The tissue sections of spinal cord injury were obtained and BBB function score was evaluated three months after the operation. The vascular density in the spinal cord scar was measured with Immunofluorescence staining. The location of syst distribution was described according to the area of cyst which was quantified by Sudan Black photo and GFAP fluorescent staining photos. Results: The average scar area in mMS group or mMS & UCBSC groups were bigger than that in medium group or UCBSC group (7.357, 7.810, 5.485 and 5.154 mm^2 respectively). However, no significant difference in average vascular density was found among four groups. The area of cyst in mMS & UCBSC group was smaller than that in mMS group, medium group or UCBSC group (0.43, 1.08, 1.27 and 1.12 mm^2 respectively). No significant difference in the percentage of non-damaged tissues was found among four groups. Conclusion: Combination of UCBSC and mMS could reduce the formation of cyst, which was the same with BBB score without affecting the new blood vessels in the scar after spinal cord transection. After spinal cord injury of rats, most cyst formed at the end of injury.

Keywords: Stem cells, mechanical micro connection system, vascular regeneration, spinal cord injury

Introduction

Because of the non-renewable nature of nerve cells and glial scar formation after injury, spinal cord injury (SCI) blocked the conduction path at the upper and lower parts of the injury. The patients showed different degrees of paraplegia, quadriplegia and other kinds of functional disorder, which seriously affected the life quality of patients [1]. Stem cell was a kind of cell with self-renewal and multi-differentiation potential, which could be trained to divide and create specialized cells. Stem cell possessed a certain transfer ability, which could arrive at injury or disease site and produce new cells. In addition to the effect of neurotrophic factors on axon regeneration, new vessels also played an important role in it [2, 3]. Some studies have confirmed that the axons grew along the direction of the new blood vessels. In addition to the scar formation after SCI, there will be a cyst or a cyst in the lesion site or near the lesion; however its formation mechanism was not very clear. SCI had effect on the regeneration of blood vessels and axis cylinder, so it would also influence neural function recovery [4]. At present, umbilical cord blood stem cells (UCBSC) could be obtained without wound or ethical issues, and widely applied in the clinical experiment. And it was confirmed that it would not induce tumor formation by transplanting UCBSC into different animal models [5, 6]. Mechanical Micro-connertor System (mMS) was a multi-channel, honeycomb-like micro system which was formed by polymethylmethacrylate (PMMA). mMS had been confirmed to

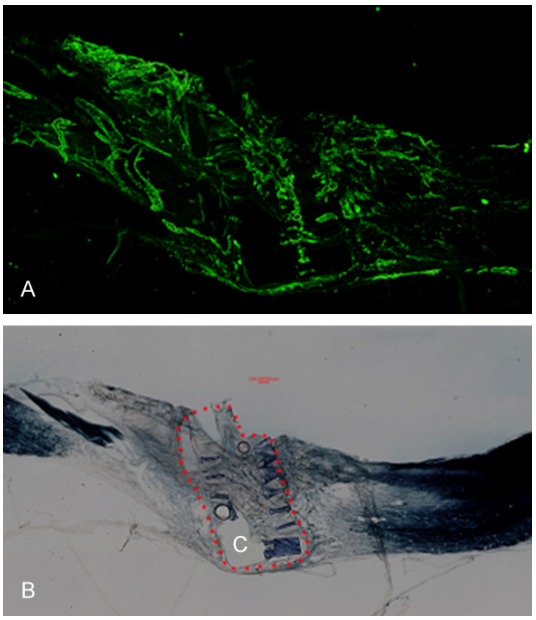

Figure 1. A: VWF immunofluorescence staining of tissue sections (the green part represented blood vessel endothelium); B: Sudan Black blank photo, which showed scars and bilateral intact tissue); C: Represented scar region formed by mMS.

be able to bridge the posterior spinal cord transection and improve the regeneration of axon and functional recovery of nerve-muscle. Based on the above points, the aim of this study was to explore the effect of UCBSC and mMS on new vessel formation and cyst capsular space and analyze the relation with Basso Beattie Bresnahan score (BBB score) of rats.

Materials and methods

Animals

A total of 60 adult female Wistar rats (average weight was 250-280 g) from Southern Medical University Experimental Animal Center. All the rats were randomly divided into 4 groups, medium group, UCBSC group, mMS group and mMS & UCBSC group. All the rats were raised at the standard condition (night time was 12 h/day time was 12 h) and adequate water condition. All the operations in this study were agreed and admitted by ethics committee of our hospital.

Construction of spinal cord injury model

The thoracic 8 (T8) lamina was removed and the spinal cord was exposed under general anesthesia using isoflurane. Scouten wire cutters were used to crosscut the spinal cord after opening the dura mater spinalis, and dura mater spinalis were sewed up after embedding mechanical Micro-connertor System. With regard to cell transplantation group, glass capillary was used to inject stem cells 2 mm at the head end and back end of the surgical incision with the depth of 0.8 mm and 1.2 mm respectively with the injection time of 4 min, and the concentration of 50,000 UCBSC/μl. The same volume of nutrient solution was injected to the control group. The ciclosporin A (15 mg/kg s.c; Novartis) was used for immunologic suppression 1 day before and 3 weeks after the operation. The antibiotic (Bayer Health Care) was given for anti-infective therapy and artificial bladder emptying for one week after the operation. Then non-steroidal anti-inflammatory drug (Rimadyl, Pfizer) was given to analgesia for 2 days. The neuromotor function of each group was evaluated according to Basso Beattie Bresnahan (BBB) score standards for every week in the 4 months after operation.

The acquisition and sections of tissue samples

The low temperature phosphate solution was perfused by heart perfusion for 2 minutes after the anesthesia, then formalin (Merck) was used to perfuse for 15 minutes. 2 cm spinal segment which included damaged areas was obtained and re-fixed at the temperature of 4°C for 24 hours, then preserved with 30% saccharose at the temperature of 4°C. The thickness of specimen section was 50 μm and 1 pc was chosen for staining analysis in an interval of 6 slices.

Immunofluorescence staining and photo collection

The primary antibody was von Willebrand (vWF) and Neurofilament (NF), the second antibody was goat anti-mouse conjugated with Alexa 488, goat anti-rabbit conjugated with Alexa 594. The fluorescence microscope (LSM 510, Zeiss) with magnification times of 4× and dark filtration was used. We chose to show vessel filter and blank filter to obtained slice blood vessels and blank Sudan Black photos, then processed by LSM photo shoft ware (Zeiss) z-stack, sharpening and other treatment finally we got the photos (**Figure 1**).

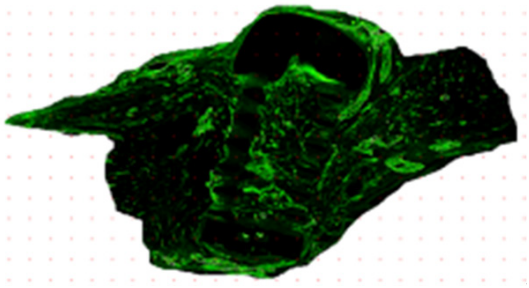

Figure 2. The image of stereological grid counting that produced by Image J software.

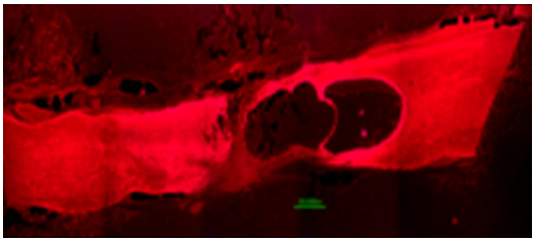

Figure 3. GFAP staining: the boundary of cystis was clear.

Figure 4. Mechanical microconnector system: ridging the stump of the spinal cord transection, we could impose the negative press to the outlet pipe and the spinal cord was sucked into the pores of the honeycomb like structure. Adjuvant therapy drugs could arrive at the different positions of spinal cord through mMS microchannel and got even distribution.

Vascular quantification

Image J software was applied to intercept the corresponding region between the scar and normal spinal cord tissues (not included cysts or cystis). The method of stereological grid counting was adopted to calculate the blood vessel density in the spinal cord scar using the formula of vessel density = the number of point on the blood vessel/total number of points in scar tissue area (**Figure 2**).

The quantization and description of cyst

According to the same methods, the slices for GFAP staining was chosen and 4 slices were selected for each animal. One slice in every 6 slices (the interval was 300 um) were taken. Photo acquisition: Sudan Black photos and GFAP fluorescence photo (**Figure 3**).

Micro connection system

Mechanical microconnector system (mMS) was a multi-channel micro system that was consisted of polymethylmethacrylate (PMMA). It was applied to rat spinal cord transection injury (**Figure 4**).

Results

Blood vessel density and scar area

The blood vessel density among four groups showed no statistical significance ($P>0.05$). However, the average scar area in mMS group or mMS & UCBSC groups were bigger than that in medium group or UCBSC group (7.357, 7.810, 5.485 and 5.154 mm^2 respectively) (**Table 1**).

Comparison of cyst area

As illustrated in **Figure 5**, the cyst area of mMS group was obviously bigger than that of other two group, medium group was the lowest. The differences had significance ($P<0.05$). The difference of the not damaged tissues area in each group was similar to the difference of the total scar area. The cyst area of chip (microconnector system) group was significantly smaller than that of other groups ($P<0.05$), and the medium group was the highest. There was no significant difference in the percentage of non-damaged tissue after removal of cyst in the scar tissues of each group (**Figure 6**).

The position of cyst and BBB score

The cyst formation of mMS + UCBSC group was obviously smaller than that of other groups (**Figure 7**). The cyst formation of simple medium group was the largest. There was no significant association between the BBB score and the corresponding cyst percentage 3

Table 1. Blood vessel density and scar area

Grouping	Culture media	UCBSC	mMS	UCBSC + mMS
Average blood vessel density	0.3228	0.3116	0.3236	0.3446
Average scar area (mm²)	5.485	5.154	7.357*	7.810*

*: P<0.05, compared with culture media group.

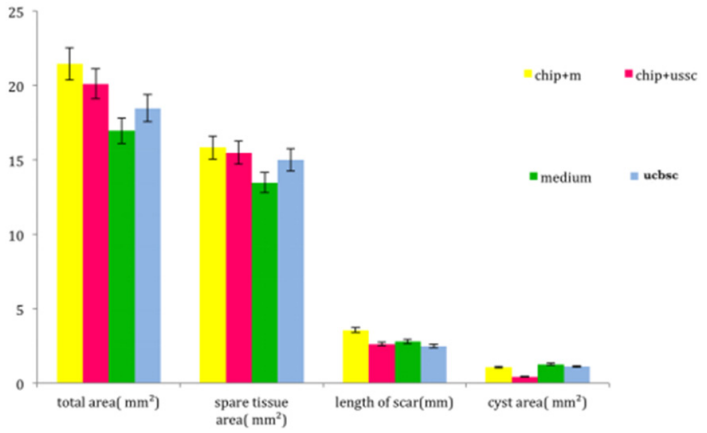

Figure 5. The comparison of cyst area for each group. Chip: micro connection system; total area: gross area; spare tissue area: non-damaged tissue area; length of scar; cyst area.

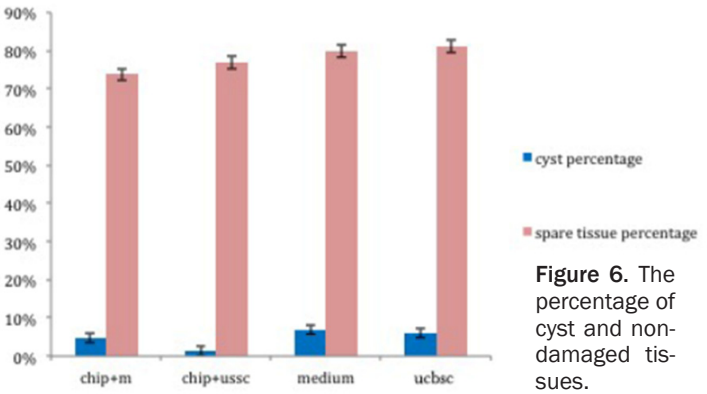

Figure 6. The percentage of cyst and non-damaged tissues.

months after operation. Most cyst were found in the caudal side of the scar (**Figure 7**).

Discussion

SCI is a kind of serious nervous system trauma, which always cause the different degrees of quadriplegia for patients or even incontinence. In recent years, researchers made gradual, systematic and deep research on SCI, but so far there is no effective clinical treatment. One of the most important reasons is the ischemia after SCI, the formed capsular space and glial scar become an insurmountable obstacle to axon regeneration [7, 8].

In all the second mechanisms of SCI, the position of vascular theory was relatively important. Severe SCI could cause decreased sympathetic excitability and fall of blood pressure, so that the spinal cord could not get the effective local blood supply [9]. Akdemir et al. found that spinal cord blood flow decreased in several hours after the injury by experimental SCI, which could last for 24 hours, and gray nucleus was the most obvious. They indicated that early central gray matter hemorrhage in the injured area by pathological examination, then gradually expand the scope and spread around. The bleeding area and its peripheral white matter occurred with clear border around the wound after infarction 24-48 hours after injury. In the previous study, we found the formation of cyst in SCI area was the marked pathological changes after SCI. Glial scar isolated the cyst from the surrounding spinal cord tissues. The astrocyte in glial scar represented as cell enlargement, the increased number and thicken of bumps. But only a small number of them got through the medial part of the scar, which meant glial scar had mechanical barrier function on nerve fibers [10-12]. So how to promote the regeneration of blood vessel after SCI and decrease the formation of cyst and glial scar were the key steps to treat SCI.

Currently, stem cell transplantation is a hot spot. Erices et al. first described cord blood-derived mesenchymal stem cells (CB-MSC) in 2000, which had a similar immune phenotype with marrow-derived stem cells. In 2004, Kogler et al. found a kind of umbilical cord blood stem cells with more differentiated potential than CB-MSC and named it as umbilical cord blood stem cells (UCBSC). UCBSC had many similarities with cord blood-derived

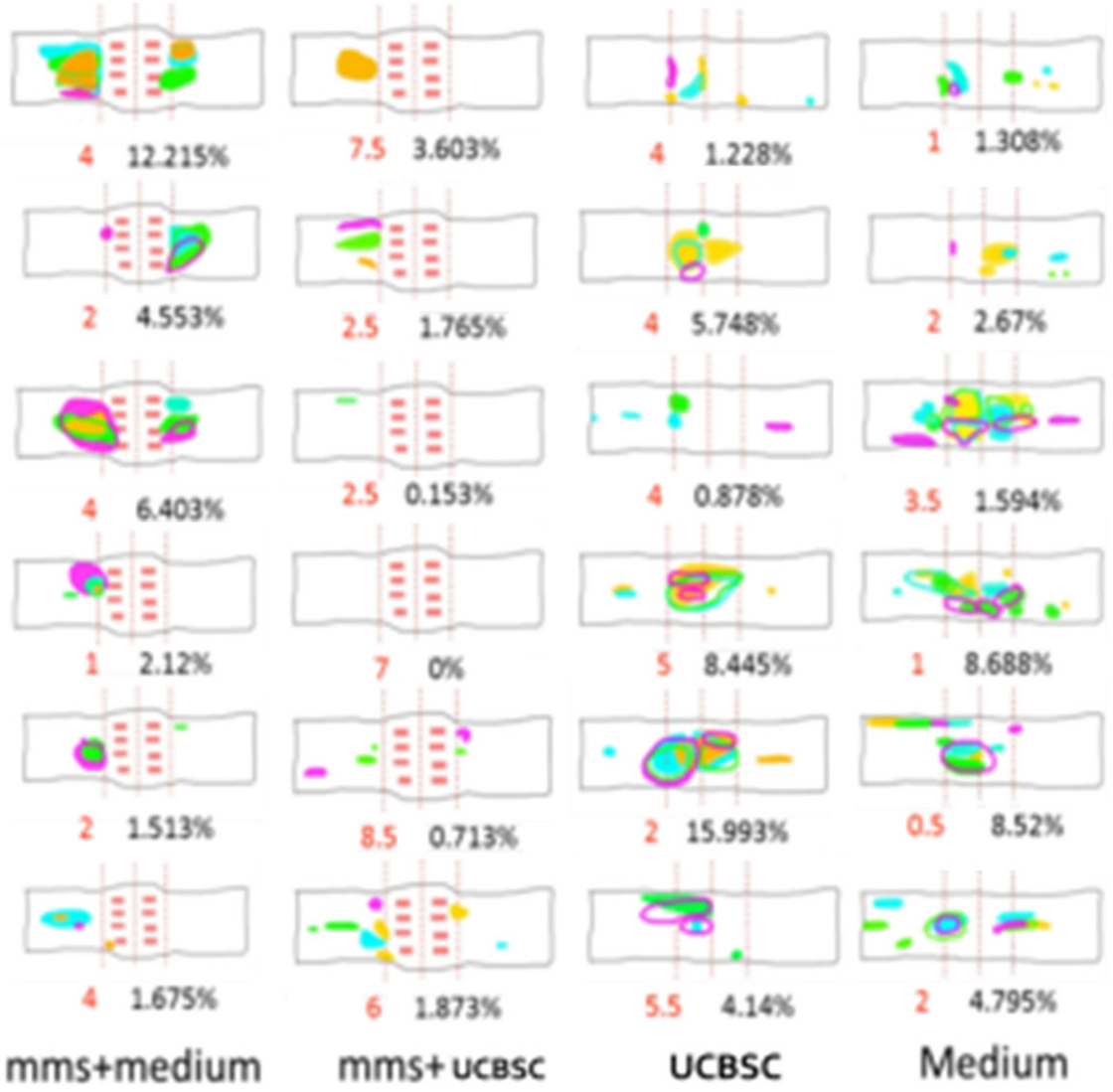

Figure 7. The gross distribution, percentage and BBB score 3 months after operation of cyst for each group (the left side of each slice was rat spinal caudal. The red number was BBB score, the percent was the percentage of the total area occupied by the average void area.

and marrow-derived mesenchymal stem cells, such as promoting the bone growth in vitro and in vivo, the cartilage formation and differentiation potential and etc, but their immunological behaviors and transcriptome sequencing were obviously different. Compared with cord blood-derived and marrow-derived mesenchymal stem cells, UCBSC terminal end of a chromosome had longer telomere and DNA repetitive sequence. It could be almost excluded in a 16-month xenoplastic transplantation tumor causing test [13-16]. UCBSC could be non invasive and effective purified, and easily amplified to meet the number of clinical applications. At present, it had been confirmed that UCBSC could differentiate into neuron like cells by specific in vitro induction, which was able to express neurofilament, B-III-tubulin, synaptophysin, NeuN and many kinds of markers. In addition, UCBSC also could express IL-1, IL-6 and other kinds of interleukin, SDF-1, HGR, VEGF and other growth factors. So it was the ideal seed cells of stem cell transplantation for central nervous system injury [17].

In the study of SCI, the use of the biological scaffold was generally made of the material with holes, which could lead medicine and nutrition factors to damage center position.

mMS made by the PMMA itself could engage the broken ends of the spinal cord and promote nerve and blood vessel regeneration. During the operating process, we did not find it could increase the bleeding from the broken ends, but it might have slight traction on the axon. The previous studies had indicated that minor movements would not have a significant impact [18]. Because of the functional disorder of blood brain barrier after spinal cord injury and the limitation in medicine, 4 micro channels pf mMS could make the medicine and the nutrients in the cross section evenly and fully and last for at least 1 week. With regards to clinical application, micro connection system was only suitable for pinal cord transection cases. It would be more valuable and significant if we can adopt PMMA system with biodegradation.

In the 4 groups of animals in this study, although blood vessel density in the scar of UCBSC & mMS group was the highest, there was no significant difference among groups. It might relate to the small quantity of slices. The results would be more reliable if we increased the number of slice and chose the continuous slice analysis. According to the position of cyst, we also found most cyst formed at the end of the injury, and this point had not been reported in the researches. But the decreased cyst could promote the recovery of neurological function after SCI, which was the same with the existing reports [19, 20].

In conclusion, stem cell has a very optimistic outlook on the treatment of SCI. Compared with other stem cells, UCBSC possesses more advantages. Mechanical micro connection system could promote rat nerve function rehabilitation level after SCI, and combined treatment with other neurotrophic factors might be an effective method of SCI treatment in the future.

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Disclosure of conflict of interest

None.

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References

[1] Konstantinov IE. In search of Alexander A. Maximow: the man behind the unitarian theory of hematopoiesis. Perspect Biol Med 2000; 43: 269-76.

[2] Kim HJ and Sun W. Adult neurogenesis in the central and peripheral nervous systems. Int Neurourol J 2012; 16: 57-61.

[3] Blair K, Wray J and Smith A. The liberation of embryonic stem cells. PLoS Genet 2011; 7: e1002019.

[4] Stemple DL and Anderson DJ. Isolation of a stem cell for neurons and glia from the mammalian neural crest. Cell 1992; 71: 973-85.

[5] Yip PK and Malaspina A. Spinal cord trauma and the molecular point of no return. Mol Neurodegener 2012; 7: 6.

[6] Garbossa D, Boido M, Fontanella M, Fronda C, Ducati A and Vercelli A. Recent therapeutic strategies for spinal cord injury treatment: possible role of stem cells. Neurosurg Rev 2012; 35: 293-311; discussion 311.

[7] Hernandez J, Torres-Espin A and Navarro X. Adult stem cell transplants for spinal cord injury repair: current state in preclinical research. Curr Stem Cell Res Ther 2011; 6: 273-87.

[8] Grabel L. Prospects for pluripotent stem cell therapies: into the clinic and back to the bench. J Cell Biochem 2012; 113: 381-7.

[9] Trounson A, Thakar RG, Lomax G and Gibbons D. Clinical trials for stem cell therapies. BMC Med 2011; 9: 52.

[10] Nogradi A, Pajer K and Marton G. The role of embryonic motoneuron transplants to restore the lost motor function of the injured spinal cord. Ann Anat 2011; 193: 362-70.

[11] Sakiyama-Elbert S, Johnson PJ, Hodgetts SI, Plant GW and Harvey AR. Scaffolds to promote spinal cord regeneration. Handb Clin Neurol 2012; 109: 575-94.

[12] Wang M, Zhai P, Chen X, Schreyer DJ, Sun X and Cui F. Bioengineered scaffolds for spinal cord repair. Tissue Eng Part B Rev 2011; 17: 177-94.

[13] Madigan NN, McMahon S, O'Brien T, Yaszemski MJ and Windebank AJ. Current tissue engineering and novel therapeutic approaches to axonal regeneration following spinal cord injury using polymer scaffolds. Respir Physiol Neurobiol 2009; 169: 183-99.

[14] Wada T, Honda M, Minami I, Tooi N, Amagai Y, Nakatsuji N and Aiba K. Highly efficient differentiation and enrichment of spinal motor neurons derived from human and monkey embryonic stem cells. PLoS One 2009; 4: e6722.
[15] Cao QL, Zhang YP, Howard RM, Walters WM, Tsoulfas P and Whittemore SR. Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. Exp Neurol 2001; 167: 48-58.
[16] Parr AM, Kulbatski I, Zahir T, Wang X, Yue C, Keating A and Tator CH. Transplanted adult spinal cord-derived neural stem/progenitor cells promote early functional recovery after rat spinal cord injury. Neuroscience 2008; 155: 760-70.
[17] Salazar DL, Uchida N, Hamers FP, Cummings BJ and Anderson AJ. Human neural stem cells differentiate and promote locomotor recovery in an early chronic spinal cord injury NOD-scid mouse model. PLoS One 2010; 5: e12272.
[18] Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Schut D and Fehlings MG. Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. J Neuro Sci 2010; 30: 1657-76.
[19] Blair K, Wray J and Smith A. The liberation of embryonic stem cells. PLoS Genet 2011; 7: e1002019.
[20] Puri MC and Nagy A. Concise review: Embryonic stem cells versus induced pluripotent stem cells: the game is on. Stem Cells 2012; 30: 10-4.