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SCHWANN CELLS - THE NEW HOPE IN URINARY BLADDER WALL GRAFT INNERVATION, SCHWANN CELLS ISOLATION METHOD.

Hypothesis / aims of study

There are several methods to induce the innervation of urinary bladder wall graft after augmentation. None of this method is effective enough to support the function of urinary bladder. Our previous study demonstrate poor innervation of chitosan urinary bladder graft [1]. Neurons from urinary bladder wall and omentium can not elongate and branch in graft because of lack of neurotrophic factors. The best source of these neurotrophic factors are Schwann cells which can be transplanted into urinary bladder wall graft. Schwann cells produce family of Neuronal Growth Factor (NGF), Neurotrophin 3 and 4 and Brain Derived Neural growth Factor (BDNF). Schwann cells also interact with axons and adopt phenotype to requirements of neuronal cells.
To transplant Schwann cells the proper amount of cells is needed which can be only obtain during *in vitro* Schwann cell cultivation. We introduce the results of Schwann cell isolation (according to Brooks method) and *in vitro* cultivation [2].

Study design, materials and methods

Schwann cells were isolated from *in vivo* degenerated peripheral nerves. During Wallerian degeneration Schwann cell proliferate and demonstrate the most activated phenotype.
Both sciatic nerve of ten adult male Wistar rats was exposed through a dorsal incision under pethobarbital anesthesia and transected at the midthigh to allow a nerve to be predegenerated. 7 days later, the distal segment of the predegenerated nerve, 20 mm in length (weighted 13- 25 g), was resected and washed two times in phosphate-buffer saline.
Twenty distal fragments of sciatic nerve were divided in five groups with four fragments each.
The nerve was cut into pieces 1 mm in length and the epineuriums were mechanically dissociated with micro forceps. Nerves were incubated with an enzyme mixture of 1mg/ml collagenase type I and 0,25% trypsine in 5 ml of DMEM (Dulbecco's Modiffied Eagle's Medium, Sigma, Germany) in 5% CO_2 at $37^{O}C$ for 2h. After incubation, the fragments were centrifuged at 2000 rpm for 10 min. and the supernatant was removed. Cell suspension from each group was suspended in 5 ml of starter medium 20% FBS (Fetal Bovine Serum).
Aproximatly 2.6 x 10^{5}cells were seeded on 25 cm^2 culture flask. After two days starter medium was change for cultivation medium with 10%FBS. After 10 days cells were immunostained for marker S-100.

Results

The cell amount isolated from four nerves was enough to establish primary culture on 25 cm^2
culture flask Prolifereted schwann cells were observed in the second day of cultivation. Spindle shapes cells were noticed (pic. 1). Monolayer can be achieved after 2 weeks of culture. Succesful rate of establishing primary cultures was 60%. Contaminations were observed in the cases of culture failure. 40-70% of cells were positive were positive for S-100 marker (pic. 2).

Interpretation of results

Method of isolation Schwann cells from rat's predegenerated peripheral nerve is effective and can delivered require amount of cells for transplantation in urinary bladder graft.

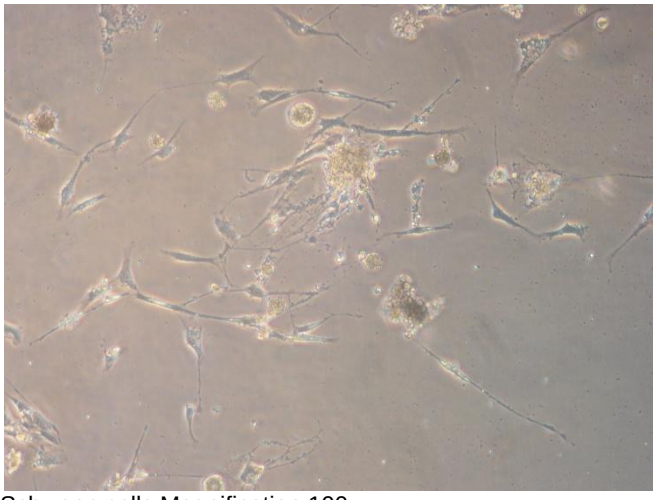
Concluding message

In the presence of ineffective methods of inducing innervation of urinary bladder wall graft after urinary bladder augumenetation Schwann cell transplantation could be promising therapy.

References

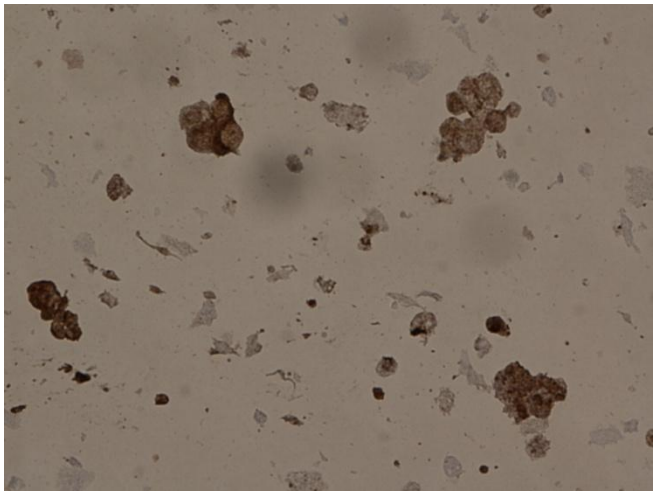
[1] Urol Int, (2008) (in press).
[2] Brain Res (1979) 165;105-108.

Picture 1



Schwann cells Magnification 100x

Picture 2



Schwan cells immunostained for marker S- 100
Magnification 20x

Specify source of funding or grant	**The research was funded by Nicolaus Copernicus University in Torun research program.**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**The local Ethical Commission in University of Technology and Life Sciences in Bydgoszcz**