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**TISSUE ENGINEERING IN THE TREATMENT OF VESICoureTERAL
REFLUX BY CULTURED AUTOLOGOUS CHONDROCYTES IMPLANTATION
IN THE RENAL TRANSPLANTATION CANDIDATES**

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Background. In patients with chronic renal failure who are candidates for renal transplantation in 2% to 8% vesicoureteral reflux (VUR) can be detected as a cause of kidney failure. Some years ago VUR grade IIb or higher in the renal transplantation candidates has been surgical corrected or nephroureterectomy has been done to prevent later complications such as recurrent urinary tract infections and pyelonephritis attacks. Our policy is to preserve candidate's native kidney and correct VUR using endoscopy. Our experience was that endoscopic correction of VUR with materials such as collagen and teflon were not quite successful. These materials can migrate or are reabsorbed by the surround tissue. Our aim was to use cultured autologous chondrocytes suspension injected under ureteral orifice for endoscopic treatment of VUR with purpose to eliminate or downgrade high grade VUR and to abandon surgical treatment.

Method. A small piece of elastic cartilage from candidate's ear is taken under local anesthesia. Cells are isolated with enzyme collagenase, seeded into cell culture vessel in liquid medium with 2% autologous serum and expanded up to $2-4 \times 10^7$. Prior implantation, cell viability is determined by flow cytometry as well as apirogenicity and sterility of cell product is tested. On the day of application, candidate's blood plasma is isolated and simultaneously with chondrocytes mixed with trombine suspension implanted endoscopically under refluxing ureteral orifice. The volume of chondrocyte suspension was at the first application 10 ± 1 ml, and at the second or third one 7-8 ml. Evaluation of the procedure was done 6-8 weeks later performing X-ray contrast

cystography. In case of insufficient correction at the first application, we can proliferate cryopreserved chondrocytes again and repeat the implantation procedure several times.

Though it is hard to define VUR grade in nonfunctional kidney we have graded VUR according to contrast cystography as follows;

I. grade: lower ureteral filling

II.a grade: ureteral and pelvicaliceal filling without dilatation

II.b grade: mild caliceal blunting without pelvis dilatation

III. grade: caliceal clubbing, moderate pelvis and ureter dilatation

IV. grade: massive ureteral and caliceal dilatation, loss of renal parenchyma

Patients. Since the year 2002 we have performed the implantation of chondrocytes in 14 candidates, six men and eight women, mean age 39,9 year (range 22 – 61). In all of them VUR grade III or higher in native kidneys was detected. Seven candidates had reflux on both sides while other seven on one side. In 4 candidates with both side reflux chondrocytes implantation need to be performed 3 times, in 3 candidates 2 times and just once at 1 candidate. Those having reflux on one side, just at 2 candidates implantation had to be performed twice in other 5 cases one implantation has been completed.

Results. At 7 candidates with both side reflux it was cured completely in two cases, in four cases it was downgraded to II. grade on one side while on the other side it was cured. Candidates having reflux on one side they were cured in four cases while in two cases VUR persisted but it was downgraded to grade II a-b. In one candidate reflux persisted unchanged. One candidate with one side VUR grade IV got one year after chondrocytes implantation septic pyelonephritis and nephroureterectomy urgently had to be done prior transplantation. At the other one with bilateral VUR which was cured on one side, but on the other side nephroureterectomy had to be performed after he was successfully transplanted, because he got recurrent pyelonephritis in reflexive native kidney. Meantime 7 candidates were successfully transplanted (4 treated previously with bilateral VUR and 3 with one side VUR) , they have no complication with regard to VUR.

Complete cure in candidates having bilateral VUR was achieved in 28,6 %, but in more than 71% it was downgraded on one side and cured on the other side. Candidates having VUR on one side were cured in 57,1%, VUR was downgraded in 28,5% and in 1 candidate treatment failed.

Conclusions. The success of endoscopic implantation in vitro cultured autologous chondrocytes under reflux ureteral orifice depends on several factors as: cell concentration, technique of implantation, neurogenic bladder disorders, repeated infections and VUR grade. Endoscopic method using autologous chondrocytes seems to be more successful comparing to other materials used previously at our institution and prevents surgical intervention in most candidates prior to renal transplantation. We consider that chondrocytes implantation has also some advantages compared to the established methods. Beside that the method seems to be successful in higher VUR grades, it can be repeated several times, chondrocytes do not migrate from the place of injection, do not change into fibrous tissue neither are reabsorbed. Cultured chondrocytes are autologous tissue, they have no impact on candidate's immunological condition and can not transmit any disease. However long-term clinical evaluation and standardisation of method will be needed to determine efficacy of this method in VUR correction or downgrading.



Fig.1. Bilateral VUR



Fig.2. After correction of VUR

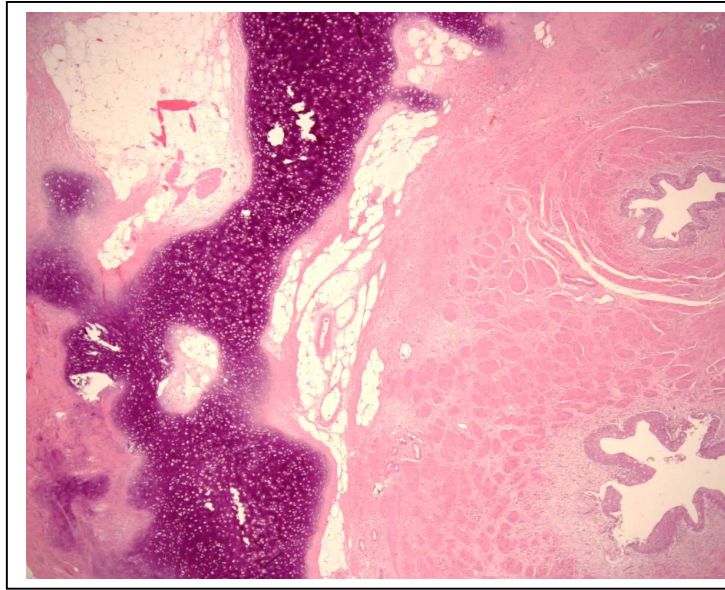


Fig.3. Chondrocytes one year after implantation under orifices