Case report

SOMATIC-CELL NUCLEAR TRANSFER: AUTOLOGOUS EMBRYONIC INTRA-SPINAL STEM CELL TRANSPLANT IN A CHRONIC COMPLETE QUADRIPLEGIC PATIENT. NEURO-ANATOMICAL OUTCOME AFTER ONE YEAR

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RESUMEN

La literatura científica informa que anualmente se producen alrededor de 180.000 casos de lesiones de la médula espinal en el mundo. Publicaciones recientes han mostrado beneficios neurológicos en casos seleccionados de personas con cuadriplejía sometidas a trasplante intralesional de células madre autólogas cultivadas de médula ósea. *Objetivos:* Este estudio de caso presenta evidencia de nivel III de recuperación clínica neurológica parcial en un paciente de sexo masculino de 32 años, con cuadriplejia completa crónica que fue sometido a transferencia de núcleos de células somáticas (SCNT, por sus siglas en inglés) y a terapia de células embrionarias por presentar lesión traumática de la médula espinal sufrida 6 años atrás. La pregunta de investigación fue: "¿Puede la terapia celular autóloga de SCNT mejorar la discapacidad motora y sensitiva de las extremidades en la cuadriplejia crónica?" *Hipótesis probada*: "La terapia celular de SCNT es incapaz de mejorar la discapacidad motora y sensitiva en casos seleccionados de personas con cuadriplejia completa crónica y no puede mejorar el resultado funcional o la independencia". *Materiales y métodos:* El trasplante celular se llevó a cabo mediante implantación quirúrgica en el área con daño medular cervical 6 años después de la lesión que dejó al paciente con cuadriplejía completa confirmada mediante examen neurológico y resonancia magnética. Después del procedimiento, se llevó a cabo la evaluación neurológica y fue evaluada la restauración de dermatomas y miotomas durante 12 meses, junto con la realización de resonancia magnética y clasificación de la American Spinal Injury Association (ASIA). *Resultados*: La mejoría neurológica se presentó en forma asimétrica en la cintura escapular y sin cambios dramáticos bilateralmente en extremidades superiores y en tronco con respecto a las funciones de las piernas evaluadas a los 12 meses. Las puntuaciones en la escala ASIA aumentaron de 29/112 a 64/112 a los 6 meses después del tratamiento y se ganó al menos un nivel de la escala ASIA. *Conclusión:* En comparación con los hallazgos iniciales, se documentó mejoría neurológica cuantificada en la cintura escapular y las extremidades superiores, entre 6 y 12 meses después del trasplante celular autólogo intralesional con SCNT en un caso de cuadriplejia crónica.

Palabras clave: *Transferencia nuclear de células somáticas, lesión de médula espinal, transplante de células madre, quadriplegía.*

ABSTRACT

The scientific literature reports that about 180,000 cases of spinal-cord injuries (SCI) occur yearly in the world. Recent publications show neurological benefit in selected quadriplegics undergoing intra-lesion transplantation of autologous cultured bone-marrow mesenchymal stem cells. *Objectives:* This case-study reports level–III objective evidence and partial neurological clinical recovery in a 32-year old-male with chronic complete quadriplegia that underwent somatic nuclear cell transfer (SCNT) and embryonic cell therapy for traumatic spinal-cord injury (SCI) sustained 6-years previously. The research question was: "Can autologous SCNT cell-therapy improve extremity motor and sensory impairment in chronic quadriplegia?" *The hypothesis* tested: "SCNT cell-therapy is unable to improve severe motor and sensory impairment in selected persons with chronic complete quadriplegia and unable to improve functional outcome or independence". *Material and methods*: Cell-transplantation was by neuro-surgical implantation into the damaged cervical cord 6-years after SCI that rendered the patient a complete quadriplegic confirmed on neurological examination and magnetic resonance imaging (MRI). Neurologic assessment, restoration of dermatomes and myotomes were evaluated post-procedurally for 12-months together with MRI, and American Spinal Injury Association grading (ASIA). *Results:* Neurological improvement was asymmetrically improved in the shoulder girdle, upper extremity bilaterally and trunk without dramatic change in leg-function at 12-months. ASIA-scales increased from 29/112 to 64/112 at 6-months after treatment and at least one ASIA-level was gained. *Conclusion:* Compared to baseline findings, measured neurological improvement was documented in the shoulder-girdle and upper-extremities, 6-12 months after intra-lesion autologous SCNT cell transplantation in a chronic-quadriplegic.

Keywords: *Somatic cell nuclear transfer, spinal-cord injury, stem cell transplantation, quadriplegia.*

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INTRODUCTION

Quadriplegia (L *quattor* four; Gk *plege* stroke) refers to the presence of paralysis of all four limbs, loss of voluntary movement and sensation, and also referred to as tetraplegia (Dorland's Illustrated Medical Dictionary, 2003; Mosby's Medical Dictionary, 1990). The medical literature indicates that the incidence of traumatic spinal-cord injury (SCI) is about 180,000 new cases in the world per-year and results in dramatic life-style changes (Fawcett et al, 2007; Sledge et al, 2013). Because spinal cord-regeneration is impaired after a high-cervical SCI, many with a complete cord-transection are rendered quadriplegic or tetraplegic for the rest of their lives. A high-level transsected cervical spinal-cord lesion severely disrupts neurophysiology, seldom heals without persisting neurological deficit, functional damage is permanent, characterised by dysfunction of the motor-system, reflexes, sensation and coordination. In some cases limited spontaneous recovery is well documented in quadriplegics (Fawcett et al, 2007; Steeves et al, 2011) and about 0.5% of tetraplegics can recover well with locomotor training after CSI (Behrman and Harkema, 2000). Motor-complete SCI persons experience fairly limited recovery (Fawcett et al, 2007).

Autologous stem cell-therapy strategies and protocols have emerged to treat high-cervical SCI (Fawcett et al, 2007; Dai et al, 2013; Jiang et al, 2013). Numerous cell-types have been investing-ated including stem cells in experimental models but translational research in this field has been difficult to interpret and implement in the clinic (Barnabé-Heider and Frisén, 2008; Goldman, 2005; Kierstead et al, 2013; Raff et al, 2012; Park et al, 2010; Novikova et al, 2011; Hagg and Oudega, 2006; Okano et al, 2003; Ota and Ito, 2003; Garbossa et al, 2006). Level-II based-evidence is now documented in the scientific literature after cell-therapy in SCI (Dai et al, 2013; Jiang et al, 2013). The technology, ethical implications, biomedical importance, application, challenges of somatic cell nuclear transfer (SCNT) with reference to application of stem cell transplantation, reprogramming of somatic cells to pluripotent state, have been documented in the veterinary and medical literature (French et al, 2008; Hwang et al, 2005; Pang et al, 2011; Lim et al, 2010; Tachibana et al, 2013; Fulka et al, 2013). In the United States of America, the National Institute of Health permitted federal funding during 2009 for research on human embryonic tissue (Burns, 2009). In the same year the U.S. Food and Drug Administration (FDA) ethically approved the first phase-1 clinical-trials using embryonic stem cells for patients with spinal cord injuries (Burns, 2009). This case-controlled study describes the neurological improvement with level-III scientific evidence, in a chronic-quadriplegic male-person that underwent autologous SCNT embryonic cell transplantation for permanent, high-level and long-segment, cervical SCI.

CASE REPORT

A 32-year old caucasian male underwent elective, autologous, intra-spinal embryonic-cell transplantation within protocol and patient consent, 6-years after sustaining a high-complete sensory and motor quadriplegia, following a diving neck-injury and confirmed by MRI-investigation (Kidson,2013). The patient was rendered a complete high level-C4 traumatic-induced quadriplegic in 2006 with respiratory problems and underwent spinal-fusion of C4/5, needing 7-weeks of ventilation until he could breathe spontaneously without tracheostomy. Rehabilitation thereafter was in a wheelchair driven by chin-control, urinary and faecal incontinence managed by catheter-diversion and oral laxatives. Over 6-years with contemporary rehabilitation, minimal spontaneous-recovery of C5 was evident, allowing partial control of the head and neck, random-assisted joystick control with lateral support, ventilation-independence and restricted shoulder and neck-movement, but sustained paralysis of the fore-arms (supported in a tray) and legs. Persisting, partially intact segments, C1-5 at 6-years, allowed limited bilateral voluntary movements of the following muscles and groups: upper trapezius, rhomboids, levator scapulae, sterno-mastoid, infra-hyoid group, and diaphragm (Moore, 1992).

Post-transplant care and neurological-assessment was performed by an inter-disciplinary rehabilitation team. Post-intervention neurological improvement was gauged by clinical neurological-examination, dermatome and myo-tome re-innervation mapping (Buehner et al, 2012; Moore,1992). MRI (performed at 1, 6 and 12 months), ASIA spinal-cord assessment for motor and sensory impairment, Loewenstein Hospital spinal-cord independence measure, Medical Research Council (MRC) scale for muscle strength (scale of 1-5), and Oxford-Scale of muscle-strength grading were scored (Buehner et al, 2012; Medical Research Council, 1981). Objective improvement of neurological impair-ment and outcome was recorded in increments over 12-months. Goal-directed intensive occupational, physiotherapy and biokinetic-therapy was instituted including movement

control, psychological recovery, mentoring of personal home-based studies for examinations and self-development, social and employment potential for rehabilitation. Posture-training was anatomically planned and assisted-pool exercises were part of post-transplantation rehabilitation. Motor-planning ability and control was facilitated on an active-passive trainer. Electrophysiolocal mapping was instituted to gauge nerve-flow in the extremities and electrical-activity up to the groin and thus below, distal or inferior to the traumatic SCI level.

Baseline testing showed chronic complete quadriplegia, near complete bilateral preservation of C5 sensation, absent sensation C6-S5, C3-4 motor-function intact, flaccid-paralysis below C4. Also, bilateral foot neuro-pathic ulceration, Loewenstein-testing indicated independence in 12% of tasks, near normal sensation C2-5, ASIA-score of 29/112, impaired sensation T1-T7, absent sensation T8-S5 and flaccid-paralysis regarding tone were demonstrated. MRI showed complete cord-transection, retraction and definitive cord-deficit at the level of C4 (*Figure 1*).

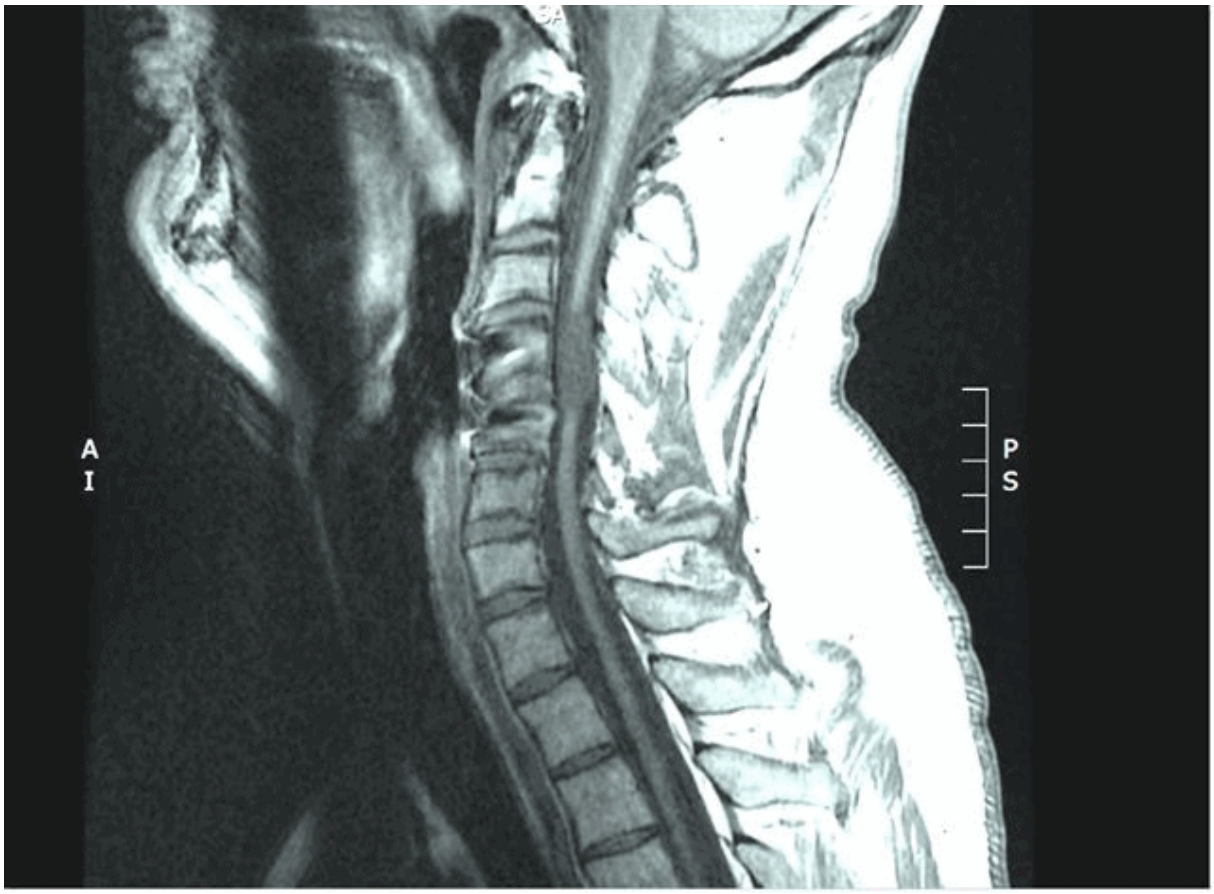

Figure1- Base-line magnetic resonance image (MRI) of traumatised cervical spinal cord in a quadriplegic: Pre-cell transplantation T1-sagittal section at the transection site and 6-years after CSI and contemporary-treatment. SCNT cell-preparation implantation was performed via a direct posterior, midline neuro-surgical access approach and after debridement of the necrotic intra-thecal glial-tissue and debris. The biological cell-transplant-construct was inserted into the traumatic cord-gap by a neurosurgeon between the two-ends of the spinal-cord (arrows). Note irreversible, skeletal injury to adjacent cervical vertebra, vertebral discs and associated spinal cord-damage.

The SCNT autologous implant-cells were generated by a commercial laboratory, and utilized pluripotent cells, because of their differentiating plasticity, as donor-karyoplast (Kidson, 2013). Autologous donor skin-biopsy was obtained to generate dermal fibroblast-cultures *ex vivo* with patient consent (Kidson, 2013). Recent published research in mammals show that it is possible to induce successful cloning by nuclear transfer of adult somatic cells

and application of oocytes (Kurd et al, 2013). The *ex vivo*, biotechnological generation of quantifiable, viable, cellular-clones using nuclear transfer technique, and resultant biological outcomes are described as a standard technique in the literature with different species(Kurd et al, 2013; Willadsen, 1986; Wilmut et al, 1977; Chesne et al, 2002; Wakayama et al, 1999; Ono et al, 2001).Researchers emphasize the use of morulas generated in the laboratory, by cleavage and tissue-culture in serum-free culture medium (Kidson,2013). After a period of culture, the autologous pleuripotential embryonic-cells are harvested, transferred and injected into the recipient's designated operative site (Kidson, 2013).

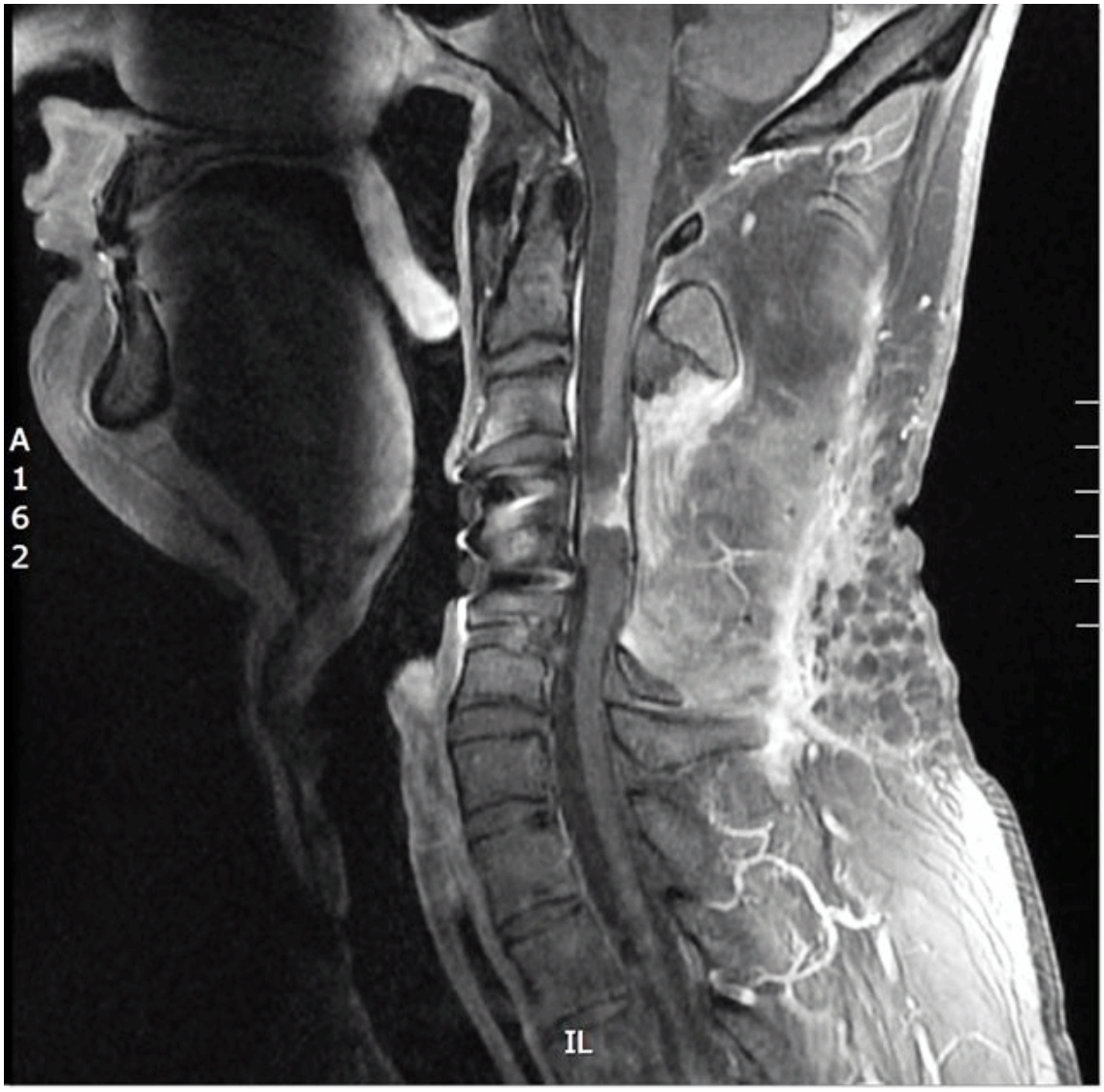

Figure 2- Follow-up post-operative MRI 6-months after SCNT cell-transplantation. T1-saggital section and gadolinium-contrast enhancement, showing spinal-cord residual ends (arrows). MRI magnification was controlled before and after image assessment to avoid amplification. Follow-up MRI of the cervical cell-transplantation site at 12-months after cell implantation in order to regenerate the damaged spinal cord, showed no evidence of teratoma formation.

The autologous cell-cultivate was inserted surgically by open cervical neuro-surgical access as a matrix or biological cell-construct, after intra-thecal debridement of about 3.5 cm of the degenerate cervical-cord and glial overgrowth, into the traumatised spinal region. Informed intervention-consent was obtained from the patient and family, after a complete protocol

disclosure including realistic potential benefit of cell-therapy, consultation and surveillance program. Similar to other international, ethical recommendations and guidelines of investigative work, referring to research in SCI. Emphasizing down-sides, outcomes and the autologous nature of donor cell-preparation consistent with modern views and thinking in medical-ethics textbooks (Moodley, 2011). Patient-ethics included due consideration to his human-rights and needs regarding self-determination as a quadriplegic and permanently restrictive life. Procedural permission was granted by the hospital-institution and health-authorities at the time to proceed. Intra-operatively objective neurologic-monitoring was employed to detect inadvertent procedural deterioration of neurology during debridement and cell-engraftment neurosurgery. Staged and interval post-procedural MRI was utilized to detect outcome, pathology and cell-induced neoplasm.

Neurological-improvement and behavioural recovery occurred steadily within the first 6-months of cell therapy sustained over 12-months in a descending and random fashion with the patient placed in the anatomical position (Dai et al, 2013; Jiang et al, 2013). Nerve conduction was measured using an electromyogram. A micro-increment nerve proliferation-rate of 1mm/day was anticipated (Walter and Israel, 1972). Parameters compared to baseline showed increased ASIA-score of 64/112 (35 point improvement), asymmetrical sensation impair-ment in all dermatomes, intact sensation C2-C5, focal sensation recovery C6-C8, improvement in sensation T3-L1 (Buehner et al, 2012). Anatomical muscle movement increments were as follows (Moore, 1992). Right-arm abduction to horizontal due to action of deltoid muscle assisted by supraspinatus (C5, 6), right upper-arm flexion against gravity due to controlled action of biceps (C5, C6), coraco-brachialis (C5, 6, 7), and brachialis (C5 and C6). Right upper-arm controlled extension due to action of triceps (C 6, 7, 8) and anconeus (C7, 8, T1). Modest function returned to the right brachio-radialis (C5, 6, 7). Slow pronation was due to the action of pronator-teres (C6, 7) and supination due to actions of biceps (C5, 6) and supinator (C5, 6). Wrist-flexion (C7- C8) was incomplete, and slower improvement occurred in left-arm function (C5-C8) with modest biceps-action, elbow-extension, supination (biceps action) and pronation (C5-6, C6-7, C7-C8) (Moore,1992). Regained trunk-stability (T4-L2) allowed unsupported sitting on a hard-surface for short periods. No controlled voluntary movements were detectable in the legs.

Modest neurological recovery parameter-gains were sustained at 12-months after SCNT cell-therapy including movements of the arms and trunk with persistent paralysis of the lower legs, bladder dysfunction and confinement need for wheel-chair and assisted render for transfers. MRI showed narrowing of the spinal injury gap (*Figure 2*) thus partially explaining some, but not all of the post-intervention related neurological recovery (arm and trunk). An interval 12-months MRI excluded the presence of a teratoma. Some function was detected in 3-fingers of the right hand, radial-side and facilitated independence. Joystick steering was now possible without lateral support. Independent feeding with a special assist-device and soft-diet was now possible with the right upper-extremity allowing more independence. Loewenstein-testing showed independence of 15% of tasks compared to base-line 12%. MRC muscle-strength grading-scales were 3 (effort) and 7 (functional assessment), but the patient remained wheelchair rehabilitated and was unable to walk, and remained graded a traumatic incomplete quadriplegic with limited neurological progress but still dependent on physical help for transfers and with daily-living (Dai et al, 2013; Jiang et al, 2013; Medical Research Council, 1981). Unsupported sitting was possible and a return of spinal-curves became more apparent. Improved motor function had returned to the upper and forearms of the upper extremities (right stronger than left) with persisting but improving finger dysfunction not observed before cell-therapy. Using the Oxford-Scale of muscle movement, it was apparent that scoring was superior in the right compared to the left-arm. Trunk action showed an ability to shift gravity on command and ability to maintain balance, not seen before SCNT cell-transplantation (Medical Research Council, 1981). Positive and quantifiable physical gains and benefits recorded by the therapists at 12-months, and attributed to cell-regeneration and embryonic-cell transplantation included: improved upper extremity muscle control and strength compared to base-line, re-learning of physical and brain-skills and motor-planning.

DISCUSSION

Although spinal-cord neuro-regeneration studies have been attempted in SCI-animal models within cell-therapy protocols, translational-research data relevant to clinical-trials in man has been questioned (Park et al, 2010; Sledge et al, 2003, Lim et al, 2010; Novikova et al, 2011).

Convincing histological-evidence is lacking that appropriate reconnection of regenerating tract-fibres and axons in the chronic-stage does occur, but research-models do show that human neural stem cells can promote functional cortico-spinal axon regeneration and synapse-reformation in injured spinal-cords of rats (Liang et al, 2006; Vierbuchen et al, 2010). Trans-differentiation of mesodermal fibroblasts to ectodermal neuronal cells is well documented (Pang et al, 2011; Caiazzo et al, 2011; Vierbuchen et al, 2010). These observations have been viewed as noteworthy as the utilization of a SCNT-biological construct in a chronic quadriplegic in this case-study, did potentially facilitate neurological improvement of the shoulder-girdle, upper extremity and trunk-function over 12-months after cell implantation. Clinical improvement confirmed by recognised objective measurements support findings from other clinical-units using other stem cell-line protocols for spinal-cord neuro-regenerative repair including cohorts with chronic SCI (Dai et al, 2013; Jiang et al, 2013; Dietz and Curt, 2007). We postulate that in this case-study the transplanted cells re-differentiated to induce a biological effect possibly by a paracrine effect. Level-II scientific evidence, and proof-of-concept in human clinical trials using non-invasive catheter-directed autologous mesenchymal bone-marrow-derived stem cells for the treatment of spinal-cord injuries has demonstrated neurological benefit and probability of deficit improvement (regarding sensory, motor and autonomic function), outcome and predictability recently in human trials with SCI (Dai et al, 2013; Jiang et al, 2013). In these recent land-mark studies, there was measureable scientific evidence that *ex-vivo* cultured bone-marrow derived mesenchymal stem cell transplantation by minimally-invasive CT-defined lesions, catheter-directed access, could induce outcome-improvement or benefit regards functional-recovery after SCI (Dai et al, 2013). Teratoma formation was not reported in these studies with short-term follow-up.

Precisely how nerve-conduction occurs in and across a spinal biological-construct or after cell regeneration is unknown and clinicians can only speculate. Evidence is lacking that stem cell transplantation can conclusively restore the spinal-cord anatomy of white and grey-matter, ventral and dorsal-horns, inter-connecting inhibitory neurones of Renshaw, ascending and descending spinal-tracts, grey-matter laminations of Rexed, or cell-bodies of the lower motor neurones as seen in man(Dai et al, 2013; Jiang et al; 2013; Dietz and Curt, 2006). Nonetheless, functional restoration has been shown with level-II studies after autologous stem cell implantation for chronic SCI (Jiang et al, 2013). The success of generating live offspring by SCNT technology is relatively low (Kurd et al, 2013). Improved cell preparation, tissue-culture standardization, including better cell-construct quantification may well improve the clinical efficacy of cell-therapy as rehabilitation option for permanent SCI. Further clinical trials will be needed to determine the place, cell-type and timing of cell-therapy in the treatment of SCI.

In conclusion, at 12-month follow-up, a positive neurological-outcome response following direct cervical stem cell implantation treatment was detectable in this SCI case-study. Measured objective improvement of sensory and motor dysfunction in the upper-extremity, and stabilization of muscle-tone in the trunk-region has also been reported by other clinicians using bone-marrow cells for repair during 2013 (Dai et al, 2013; Jiang et al, 2013). Partial and asymmetrical arm, fore-arm muscle and trunk-function improvement was thought after 6-years of SCI in this case-study, not to be attributable to induced spontaneous recovery of resident spinal-cord or thecal mesodermal cells, a placebo-effect or coincidence (Jiang et al, 2013; Dai et al, 2013; Fawcett et al, 2007). Further controlled human-trials are needed to establish proof-of-concept and benefit of cell therapy in CSI.

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