SUPPLEMENTARY MATERIAL

Bone marrow aspiration and mononuclear cell (MNC) separation. Bone marrow (BM) was aspirated from one location at the posterior iliac crest under aseptic conditions in the operating theatre using a 14-gauge, 40 mm needle (Sterylab, Milan, Italy). The procedure was performed under local (group A) or under general anaesthesia (groups B and C). In group A, 60 ml of BM was aspirated, which was considered the maximum amount that can be aspirated under local anesthesia. Following separation, a mean mononuclear cell (MNC) dose of 0.89 $\times 10^8$ MNCs (SD 0.38) was obtained. In groups B and C, a MNC dose of 4.0 $\times 10^8$ MNCs and 8 $\times 10^8$ MNCs was anticipated, respectively. In order to get these cell concentrates, 350 ml and 650 ml of BM was aspirated under general anaesthesia for groups B and C, respectively. Due to ethical considerations, in these two patient groups BM aspiration was performed in combination with a modified Steindler procedure, which is our standard secondary surgical procedure with a flexion deficiency of the elbow.

BM was collected in flasks containing Hanks balanced salt solution and heparin and was then separated over a density gradient (Ficoll isopaque specific density (SD) 1.077 g/cm^3). The MNCs were harvested and washed in 0.9% NaCl with 0.5% human serum albumin (RVG 16910; Sanquin, Amsterdam, The Netherlands). The BM-derived MNC doses were concentrated in a volume of 10 ml. The Ficoll isolation procedure was performed in a clean-room facility according to good manufacturing practice (GMP) and took approximately three hours.[1,2]

MNC injection. BM-derived MNCs were injected in the biceps brachii muscle. The 20 injections were centred on the largest diameter of the muscle belly at a distance of approximately 0.5 cm × 0.5 cm from each other.

With each injection, a volume of 0.5 ml MNCs was injected at a standard injection depth in the muscle belly (which was palpated manually) of 0.5 cm using a 26-gauge needle (Becton Dickinson, Breda, the Netherlands). The same surgeon, who also performed the tendon transfer, performed these injections (RN).

Clinical functionality. In order to maximise reliability and validity of the range of movement (ROM) measurements, the arm was kept in a standardised position (0 anteflexion, 0° abduction, 0° rotation of the shoulder). Force measurement of the injured biceps muscle was also performed in a standardised arm position (0° anteflexion, 0° abduction, 0° rotation of the shoulder and 90° elbow flexion). All measurements were performed by the same examiner (RN).

CT scan. The Aquilion 64-slice CT scanner was used (Toshiba Medical Systems, Otawara, Japan). The scanning parameters were set at 135 kVp and a detector pitch of 53 (pitch factor 0.828). A soft-tissue filter and boost three-dimensional (3D) artifact suppression was used, producing a 512 × 512 matrix of 1-mm thick slices (slice overlap: 0.2 mm). A total of 50 consecutive multiplane reconstruction (MPR) images were computed by reconstructing images in the coronal and sagital plane so that in every patient axial, slices were used for analysis, thereby correcting for oblique slices. As observed, the largest transversal diameter of the biceps brachii muscle was shifted from the midpoint of the humerus to the distal 2/3 point of the humerus. Therefore, 50 consecutive images were selected from 25 mm superior and 25 mm inferior to this distal 2/3 point of the humerus. This was measured using the most superior part of the humeral head and the most superior part of the olecranon. Images were first recalibrated for air (-1000 HU) based on samples outside the patient. Subsequently, the triceps muscle and the biceps brachii muscle were manually outlined as separate regions of interest (ROI). All pixels containing bone tissue were automatically excluded from the segmentation by applying a threshold value of 200 HU. A histogram was constructed from all pixels within the outlined ROI to calculate the mean muscle density (MMD).[3]

Quantitative needle electromyography. Quantitative needle electromyography (EMG) was performed using the Medelec Synergy EMG system (Oxford Instruments, Oxford, United Kingdom). The EMG signals were recorded with filter settings of 5 Hz for high pass and 10 kHz for low pass. For the biceps brachii muscle, the electrode was positioned in the middle of the muscle belly. For the brachialis muscle, the electrode was positioned medial to the biceps muscle at the level where the latter muscle adheres to the tendon. When the EMG signal was crisp, no further adjustment of the electrode position was made and motor unit potentials (MUPs) during voluntary contraction were recorded for 30 seconds. Potentials that were regarded as belonging to unique motor units but were judged to represent a motor unit already collected were omitted, as well as those with a noisy baseline or other artifacts. Among the remaining MUPs, the automatic duration cursor settings were manually corrected if necessary. If the duration cursor was changed, the amplitude and number of phases of the MUPs were recalculated before analysis.[4] All analyses and re-analyses were performed by the first author (SH) and an experienced neurophysiologist (JvD) blinded to the patient characteristics.

Histochemistry and immunohistochemistry of muscle biopsy. A biopsy of the injured biceps muscle was performed at the site of the MNC injection using a forceps with two sharp-edged jaws (Blakesley Conchotoma; DK Instruments, West Bengal, India).[5] The muscle biopsies before and after cell therapy in group A were taken under local anaesthesia at the outpatient clinic. The muscle biopsies before cell therapy in groups B and C were performed under general anaesthesia in combination with

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the modified Steindler procedure. The muscle biopsies after cell therapy in groups B and C were taken under local anaesthesia at the outpatient clinic.

For Masson's trichrome staining, sections were incubated subsequently for 60 minutes at 56°C in Bouin's fluid (640960; Klinipath, Duiven, the Netherlands), 15 minutes in 1% Biebrich Scarlet (03336; Brunschwig Chemie, Amsterdam, the Netherlands), 15 minutes in 5% Tungstophosphoric acid (1005830100; Merck, Damstadt, Germany) and 10 minutes in 2% light green SF yellowish (C.I. 42095, 115941; Merck) resulting in a green staining for fibrosis and red staining for muscle fibres.

For immunohistochemistry, the primary antibodies monoclonal mouse anti-human Pax7 (1:20, 352-523; Developmental Studies Hybridoma Bank, Iowa City, Iowa), monoclonal mouse anti-human CD56 (1:80, clone NCAM16.2, 345811; BD Biosciences, Erembodegem-Aalst, Belgium), and polyclonal rabbit anti-human von Willebrand Factor (vWF) (1:4000, A0082; Dakocytomation, Heverlee, Belgium) were used. Sections were pretreated by boiling for 20 minutes at pH 6.0 in 10 mM citric acid buffer (C0759-500G; Sigma-Aldrich Chemie, Zwijndrecht, the Netherlands) (Pax7 and vWF) or at pH 9.0 in 1 mM ethylenediamine-tetraacetic acid disodium salt buffer (1073; Baker, Deventer, the Netherlands) (CD56) for antigen retrieval. Next, sections were incubated for 60 min in 5% normal goat serum (NGS) (X0907; DakoCytomation, Heverlee, Belgium) and 1% bovine serum albumin (BSA) (A9647-50G; Sigma-Aldrich Chemie) in phosphate buffered saline (PBS) to block nonspecific binding. For Pax7, 0.3% Triton X 100 (T8787; Sigma-Aldrich Chemie) and 0.01% hydrochloric acid (1003171000; Merck) was added to the blocking solution. Sections were incubated with the primary antibodies overnight and subsequently for 20 minutes in 0.3% hydrogen peroxide (1072090250; Merck) in PBS to block endogenous peroxidase. Next, the sections were incubated for 60 minutes with the biotinylated secondary antibody rabbit anti-mouse (1:200, E0464; DakoCytomation) (Pax7, CD56) or goat anti-rabbit (1:200, E0432; DakoCytomation) (vWF). In order to visualise immunolabelling, sections were incubated for 30 minutes with horseradish peroxidase labeled avidin-biotin complex (Vectastain Elite ABC kit, PK6100; Brunschwig, Amsterdam, the Netherlands) and subsequently with diaminobenidine (DAB liquid, K3468; DakoCytomation) as chromogenic substrate resulting in a brown precipitate. Finally, sections were counterstained for one minute with Harris' haematoxilline solution (1092532500; Merck), dehydrated and mounted on Micromount (1731; Surgipath Europe, Peterborough, United Kingdom).

Muscle analysis. The area of fibrosis was analysed in the Masson-trichrome stained sections by dividing each pixel into three color components (hue, saturation, intensity). The threshold was defined and kept constant throughout the analysis. The percentage of green fibrotic area was measured.

The haematoxylin and eosin (H&E) stained sections were used to measure the myofibre diameter and the percentage of centronucleated, regenerating myofibres. In order to measure the myofibre diameter and the percentage of centronucleated myofibres, only transversely cut myofibres were measured. In order to compensate for myofibres that were partially longitudinal the minimal diameter was calculated. The Pax7 and CD56 stained sections were used to calculate the percentage of myofibres containing a Pax7 positive cell and percentage of CD56 positive myofibres respectively. The vWF sections were used to assess the number of capillaries around each fibre. All transversely cut capillaries were counted. If a capillary was sectioned longitudinally, it was counted as one each time it crossed a junction between three or more muscle fibres.

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