Mesenchymal Stem Cell Secretome Improves Tendon Cell Viability In Vitro and Tendon-Bone Healing In Vivo When a Tissue Engineering Strategy Is Used in a Rat Model of Chronic Massive Rotator Cuff Tear

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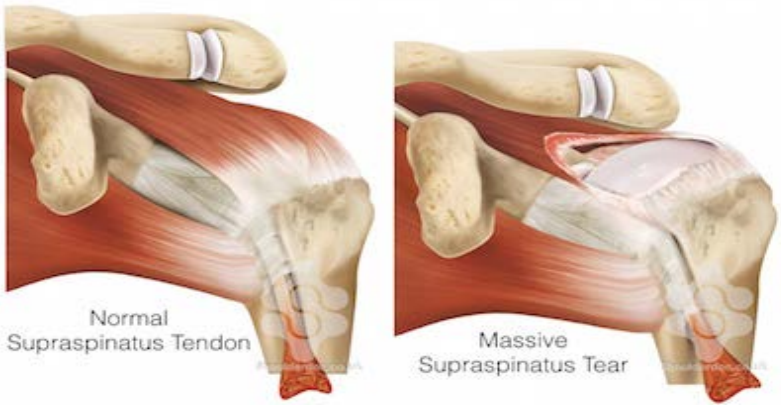
Background

Massive rotator cuff tears (MRCTs)

Background:
Massive Rotator Cuff Tear

- Greater than 5cm
- Involves supraspinatus and infraspinatus components, may extending to subscapularis and teres minor
- 20% of all cuff tears, 80% of recurrent tears



https://www.shoulderdoc.co.uk/article/1692

- Therapy:
  - Deltoid rehabilitation programme
  - Injections
  - Tendon repair with Orthobiologic materials
  - Muscle Transfer procedures
  - Arthroscopic Subacromial Decompression, Debridement and Biceps Tenotomy
  - Inspace Balloon procedure
  - Arthroscopic Superior Capsular Reconstruction (SCR)
  - Reverse Shoulder Replacement

Background:

- Failure rate for tendon bone healing: up to 94% at long term follow up
- Related to: muscle-tendon degeneration, reduced cellular activity, decreased tendon healing potential

- **MSCs secretome**: growth factors, cytokines, vesicles

  -> immunmodulatory, antiapoptotic activities
- **Human hair keratin:** tissue regeneration, repair applications
- **Electrospinning:** fibrous matrices to mimic extracellular matrix

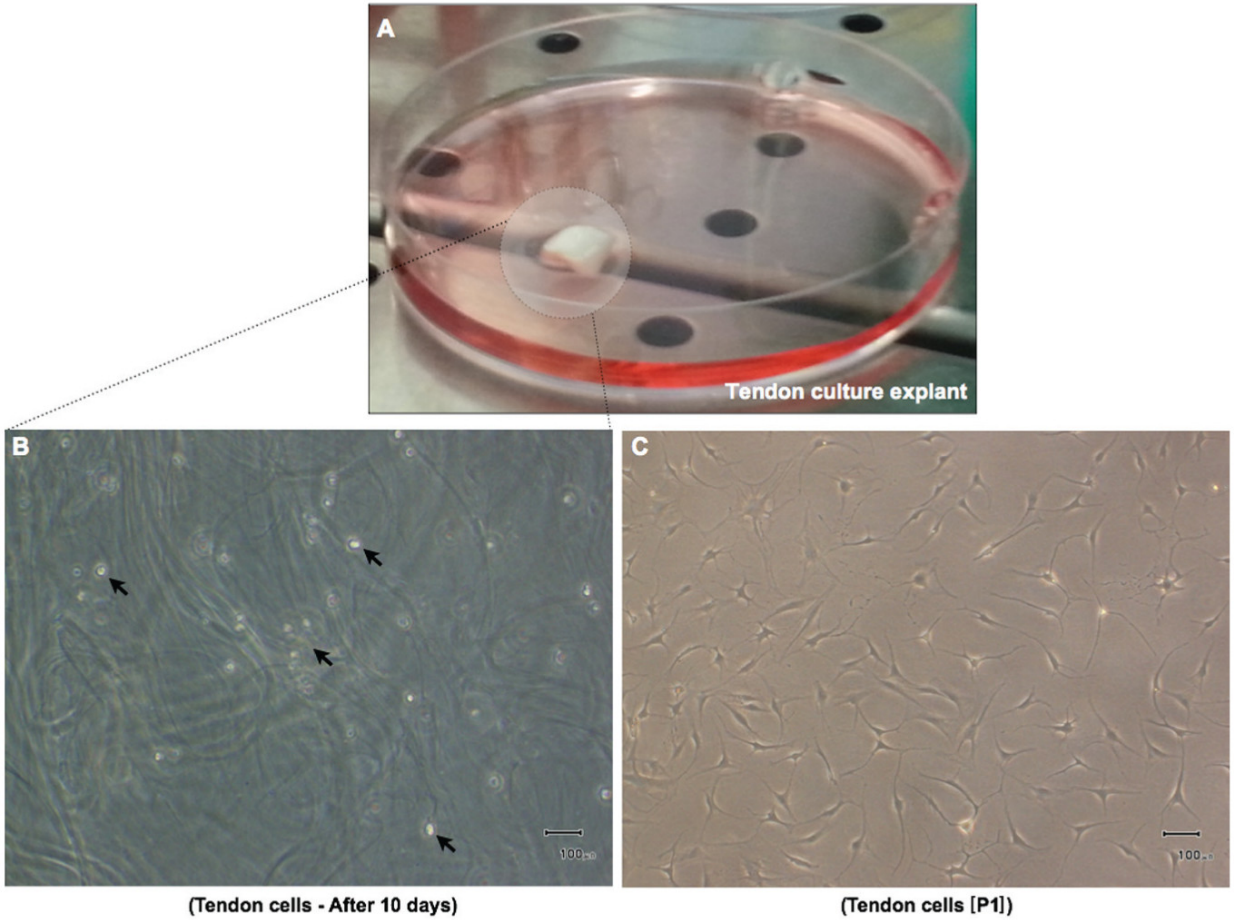
Methods

Methods: Cell preparation

- Primary Cultures of hTCs
  - *Macroscopically intact intra-articular portion of the long head of the biceps from 3 female patients (mean age, 62,3 years)*
- Preparation of hMSCs and Collection of CM
  - 5000cell/cm^2, 24 hours incubation
- Incubation of hTCs With hMSC-CM, Cell Viability Assessment and Immunocytochemistry
  - 5x10^4 cells per well incubated with CM (n=3) for 7 days (50% CM volume renewed at day 4)
  - Density and viability were assessed by MTS testing and immunocytochemistry
  - Primary antibodies: decorin, collagen type 1 and type 3

Methods:

migration



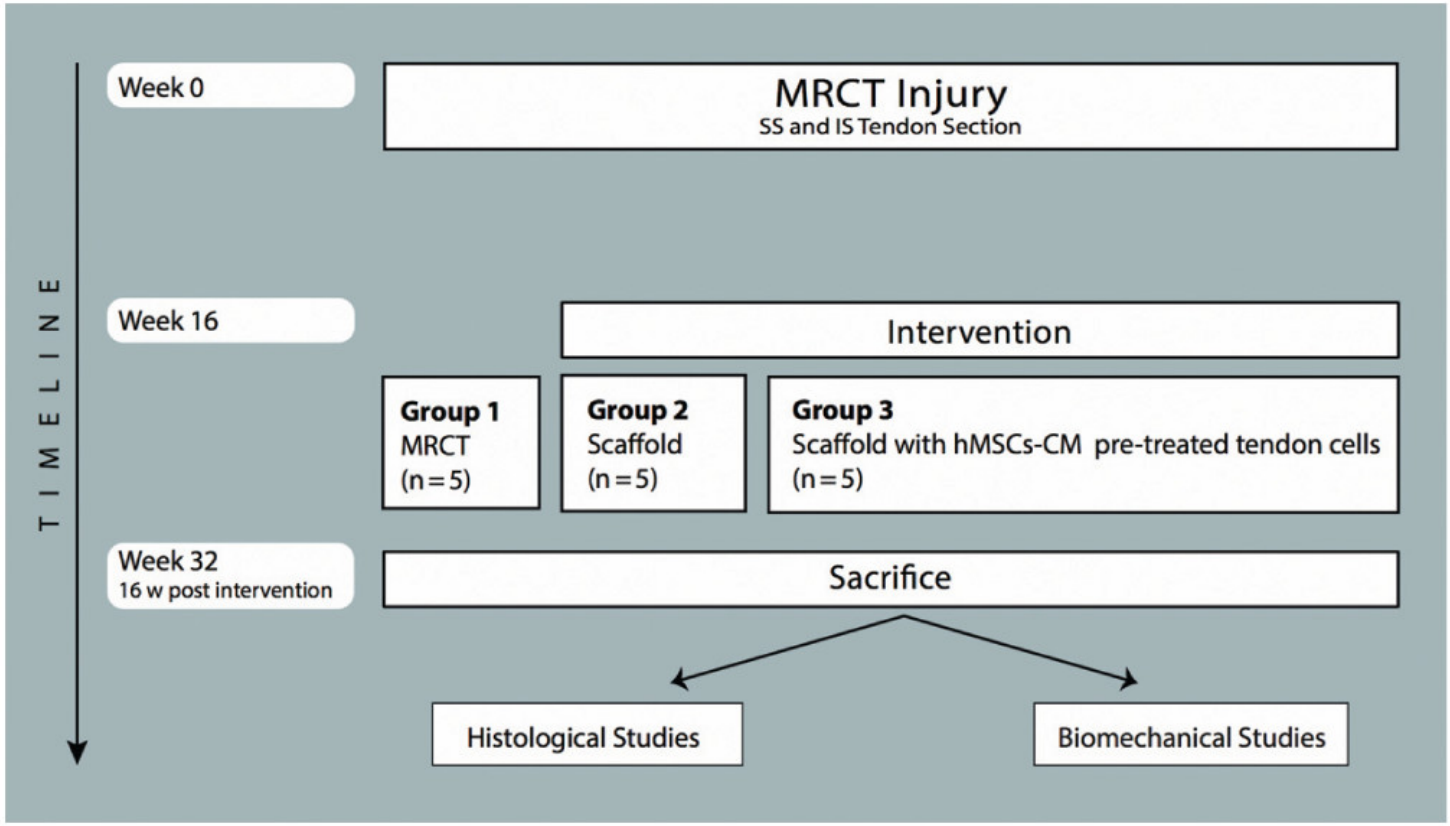
Methods: Scaffold preparation

- Electrospun Keratin Matrix Preparation
  - Keratin extraction (sodium sulfide)
  - Electrospinning
- Scanning Electron Microscopy: x2000 magnification

- Effects of hMSC Secretome on hTCs in 3D Environments
  - hTCs at density of 2x10^2 cells per scaffold onto electrospun keratin matrices (14 days, every 3 days medium change)

Methods:

- In vivo:



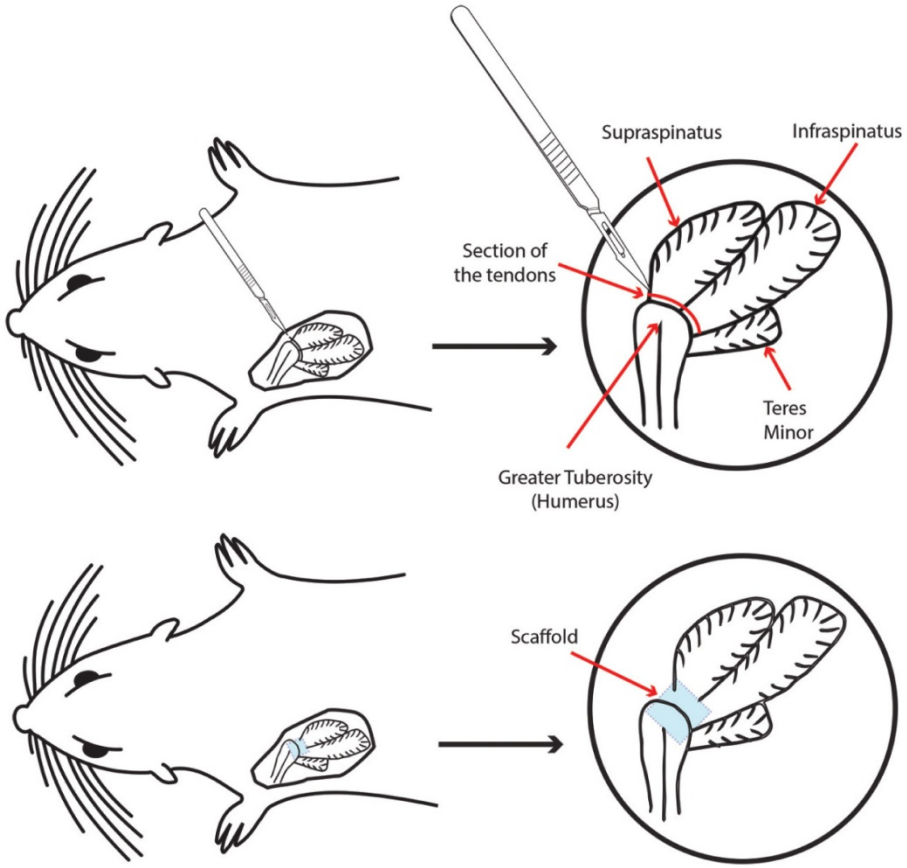
Methods:

- In vivo:
  - MRCT: N= 15 Wistar-Han rats
    5: no scaffold
    5: scaffold only
    5: scaffold
    seeded with hMSC-CM-pretreated hTCs
    Internal Control: 3 uninjured rats

  - Tests: Supraspinatus and infraspinatus muscle-tendon units together with 1,5cm of the proximal humerus
  - Histological evaluation: tendon maturing score by Ide et al
  - Biomechanical (Zwick/Roell) evaluation
    - Maximum load to failure (Fmax,N)
    - Elongation at maximum load (DLtot,mm)
    - Total elongation at failure (DLtot,mm)
    - Stiffness (N/mm)

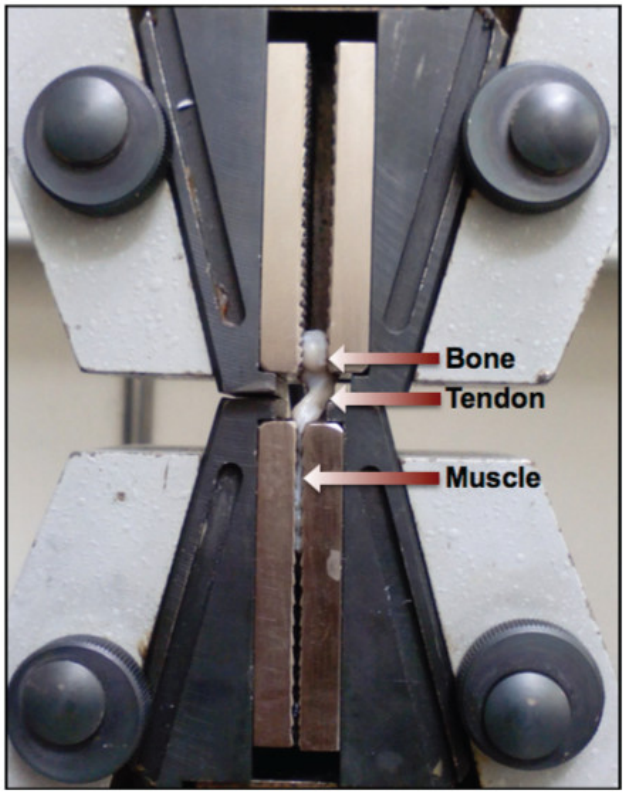
Methods:

- In vivo:



Methods:

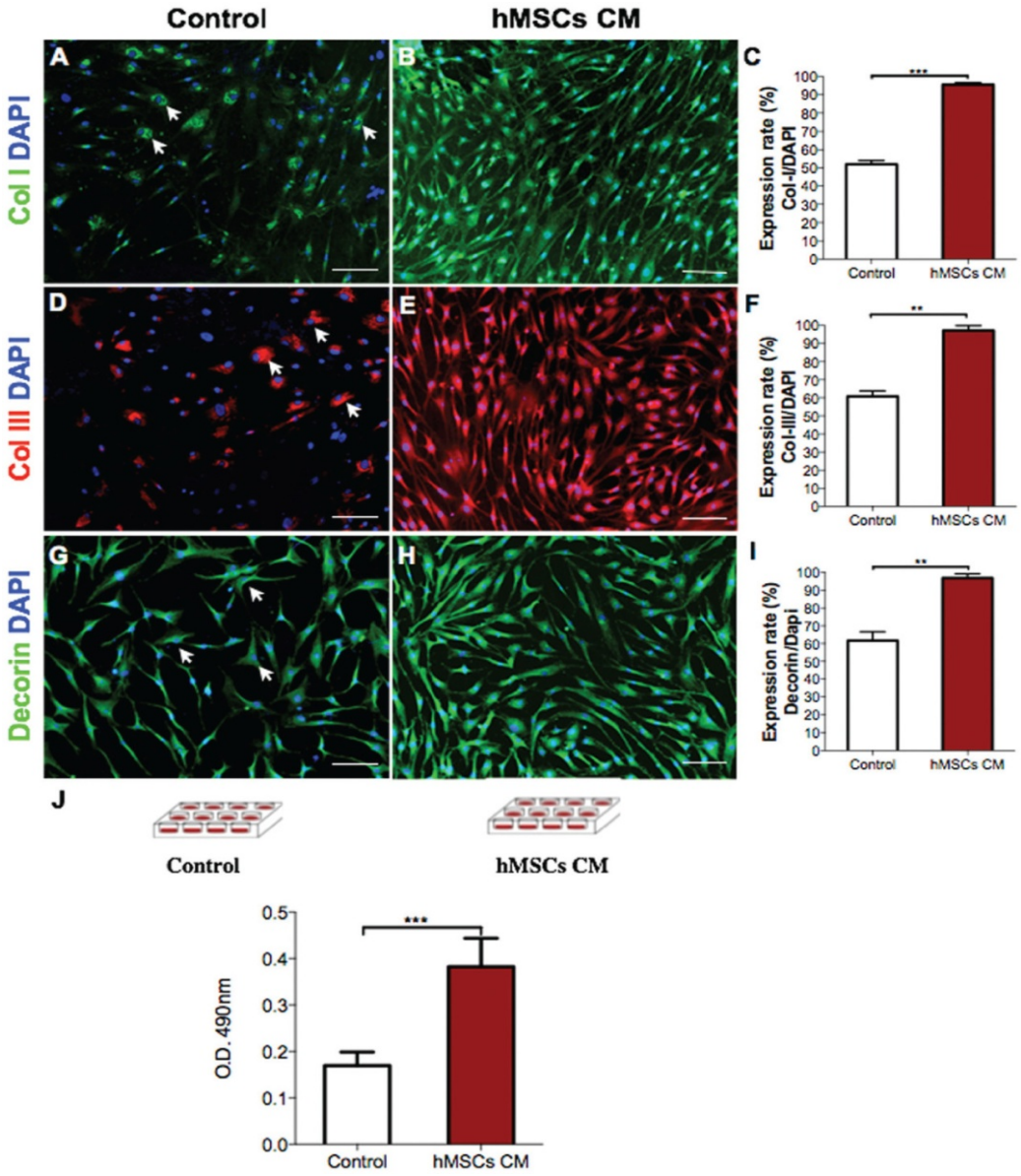
- In vivo:



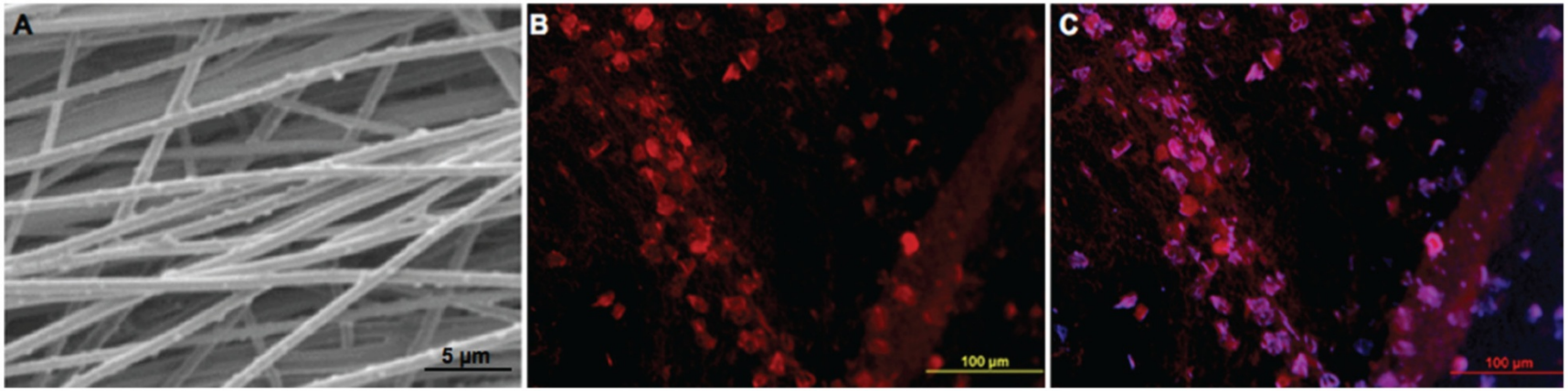
Results

Results:

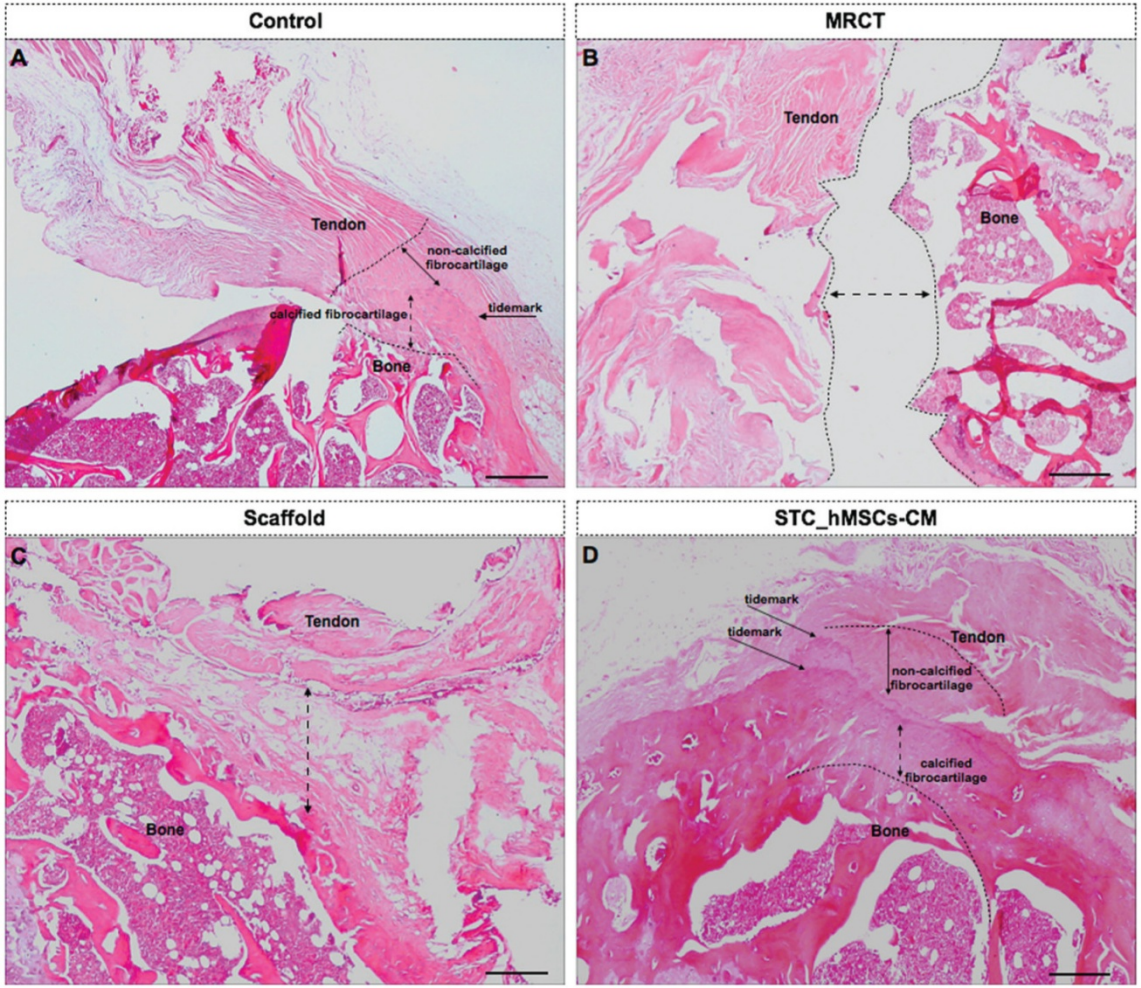
morphological analysis and immunofluorescence staining of hTCs after 7 days of incubation with hMSC secretome



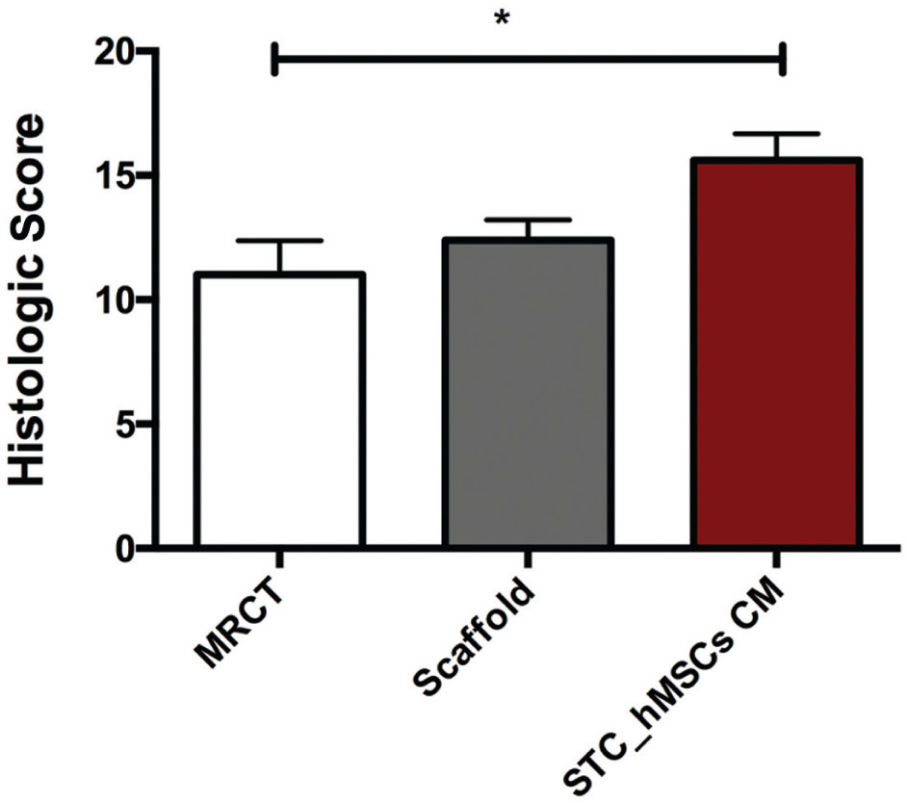
Results:
Scanning electron microscopy images



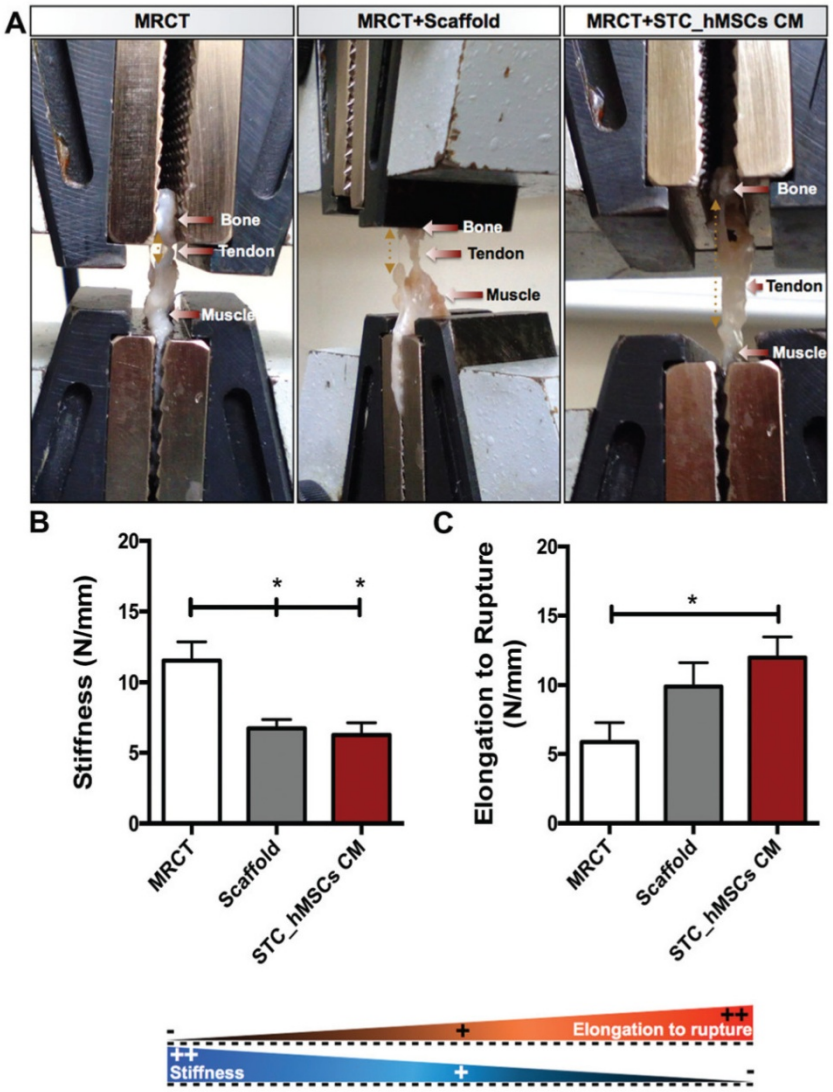
Results:
Histological analysis of rotator cuff tendon-bone interface



Results:
Ide et al modified tendon maturing score



Results:
Analysis of stiffness and total elongation to rupture



Discussion

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- The hMSC secretome-preconditioned hTCs improved the healing process without any surgical reattachment of the torn tendons
- Tendon stem or progenitor cells are proposed as a potential approach to tendon regeneration
- First description of hMSC secretome as a pretreatment for this purpose
- Known trophic factors for tendon cells in the MSC secretome:
  interleukin6, decorin, and biglycan
- „failure in continuity“

- Limitations: induced disease
  single time point for all in vivo analyses
  no surgical reattachment

Discussion

- Further limitations:
  - hMSC and hTCs
  - In vitro: cut not a tear
  - Scaffold with hTCs without pretreatment

Conclusion

- In vitro: hMSC secretome could increase phenotype, density and viability of hTCs

- In vivo: preconditioning htCs with the hMSC secretome in a tissue engineering strategy yielded improved histological healing of rotator cuff Tbi when compared with no intervention, although obtained tissue regeneration remained incomplete

Thank you for your attention