The introduction of human Mesenchymal Stem Cells to clinically available nerve substitutes

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Aims:

1. Examine the viability of MSCs when introduced to segments of the Avance Nerve Graft and the NeuraGen Nerve Guide
2. Examine the capability of MSCs to be dynamically seeded onto the surface of the Avance Nerve Graft and the NeuraGen Nerve Guide, determine optimal dynamic seeding durations and compare the seeding efficiencies of both groups
3. Determine the distribution of the MSCs after dynamic seeding

Methods:

MSC viability
An MTS assay at different time intervals measured the cell viability after a 2mm segment of the Avance Nerve Graft or the NeuraGen Nerve Guide was introduced to their well.

Seeding distribution
The distribution of MSCs was evaluated by:
- Live/dead and Hoechst stains of the surface of the nerve substitutes
- Hoechst stains of cross-sectional segments of the nerve substitutes
- Scanning Electron Microscopy of the nerve substitutes

MSC seeding efficiency
MSCs were seeded with a dynamic seeding strategy that preserved the inner ultrastructure of the nerve substitutes.
- 1 million MSCs where added to a conical tube containing either a 10mm segment of the Avance Nerve Graft or a 10mm segment of the NeuraGen Nerve Guide.
- Rotation of the conical tubes in a bioreactor for either 6, 12 and 24 hours
- Cell counts of the supernatant provided cell counts of the attached cells

ANOVA analysis to determine differences between and within groups

Results:

MSC viability
The viability of MSCs was not influenced by the presence of both nerve substitutes.

Seeding distribution
Live/dead stains and Hoechst stains on all time points showed a uniform distribution of viable MSCs over the entire surface of both nerve substitutes. Cross-sectional sections revealed that the MSCs were absent on the inside of group I, but were present on the inside of group II.

MSC seeding efficiency
For group I, a seeding efficiency of 18.23% was obtained after 6 hours, increasing to 66.46% after 12 hours (p<0.001) after which the efficiency decreased to 59.90% after 24 hours (p=1.00). For group II, the seeding efficiency increased from 52.08% after 6 hours to 94.17% after 12 hours (p=0.009) and decreased to 52.50% after 24 hours (p=0.009). Seeding efficiencies were significantly higher for group 2 after 6 and 12 hours of seeding (p=0.007 and p=0.025).

Conclusion:

1. The viability of MSCs is not influenced by the presence of both nerve substitutes.
2. Viable MSCs can be seeded on both nerve substitutes without harming the inner ultrastructure; 12 hours is the optimal seeding duration.
3. MSCs were seeded on the surface of both nerve substitutes in a uniform matter.
4. MSCs only migrated into the NeuraGen nerve guide during dynamic seeding.
5. Our methods have great clinical potential to improve and individualize peripheral nerve repair.