CHIEF SCIENTIST OFFICE

CODE: TCS/19/22

RESEARCH PROJECT BRIEFING

GMP compliant OM-MSCs retain a pro-myelinating phenotype

AIMS

The intended aims of this project were to:

- Develop a protocol for the derivation and expansion of specialised cells called olfactory mucosa mesenchymal stromal cells (OM-MSCs) thought to promote repair of central nervous system (CNS) disorders that was compliant with good manufacturing practise (GMP), standards necessary for a medicines manufacturer must meet in their production for a potential clinical trial.
- Determine key release criteria for these cells as a therapeutic product.
- Evaluate stability of cell products after freezing and storage.
- Confirm these cells retain their therapeutic properties and are safe to administer, using appropriate measurements in a dish or in a model of CNSdisease.

KEY FINDINGS

- OM-MSCs can be derived from human nasal tissue using a purely mechanical processing method. They grow well in a dish lacking animal-derived components, and meet the International Society for Cell & Gene Therapy (ISCT) minimal criteria for MSC identity within one passage, with no need to isolate and purify cells using a machine that isolate and purifies cells called a flow cytometry (FC).
- Antibiotics have proven dispensable, with endotoxin levels (indicative of bacterial contamination) below the limit of detection when tested in an NHS QC lab without using antibiotics.
- With a lack of animal derived components involved in their derivation and maintenance, and no requirement of antibiotics to maintain sterility, these can be considered GMP compliant.
- GMP compliant OM-MSCs retained their previously demonstrated classical MSC phenotype as well as their characteristic high expression of nestin and CXCL12.
- They retain their ability to promote myelination of neural cells in a dish, and in a mouse model of multiple sclerosis.
- They also maintain all of these properties when recovered from deep-freeze storage.

WHAT DID THE STUDY INVOLVE?

Many CNS disorders (such as multiple sclerosis (MS)) feature demyelination, where nerve processes loose their protective insulating sheath (myelin). This results in nerve cells losing their ability to function properly. Unique cells (called OM-MSCs) can be readily generated from human nasal biopsy tissue, and have previously been shown to promote the formation of myelin around nerve cells in a culture dish. They also do this in a mouse model of MS, suggesting they have potential as a cell therapy for MS (and similar diseases) in people.

We developed a new method for making OM-MSCs that was GMP compliant, meaning they are far closer to being usable in a clinical trial. Previously, making OM-MSCs involved various animal derived products and required antibiotics to maintain sterility making them potentially dangerous to use as a medicine. We've developed a protocol which removed or replaced these and produced a homogenous product without complicated steps to purify the cells.

We used multiple techniques to confirm MSC identity of these cells. These included flow cytometry (FC) to look at the proteins on their surface or inside them, and assays that turned these cells in to fat and bone cells (a key feature of MSCs). Their ability to promote myelination of nerve cells in a dish was assessed, and enzyme-linked immunosorbent assays (ELISAs) used to check for CXCL12 secretion (a key protein in this process). This was also assessed following deep-freeze storage and recovery. Finally, their effectiveness in treatment of an animal model of MS (experimental autoimmune encephalitis, or EAE) was evaluated.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

Assessing OM-MSCs with FC, we see that while there are non-MSCs during the initial outgrowth phase ('P0', Fig.1a i-iii) , these disappear following a single 'passage'* ('P1', growth in a dish, Fig.1b i-iii). They express ISCT minimal criteria of >95% expression of CD73, CD90 and CD105 (Fig1,b i&ii) for identification as MSCs. They also robustly express nestin (Fig1.b ii), a protein that OM-MSCs make more of compared to other MSC types. While CD271 was the protein used to purify OM-MSCs previously, it seems that between P0(Fig1.a iii) and P1(Fig1.b iii) it's expression is lost. This suggests that sorting by CD271 selection may be unnecessary. By selecting a single marker (CD105), we can compare between P0-3 (Fig1.c i) or between sorted and unsorted cells at P1 (Fig1.c ii). While there was a difference between P0 and later passages, no differences were seen between sorted and unsorted cells. In other words, a homogenous population of OM-MSCs can be obtained by growing the cells in dishes.

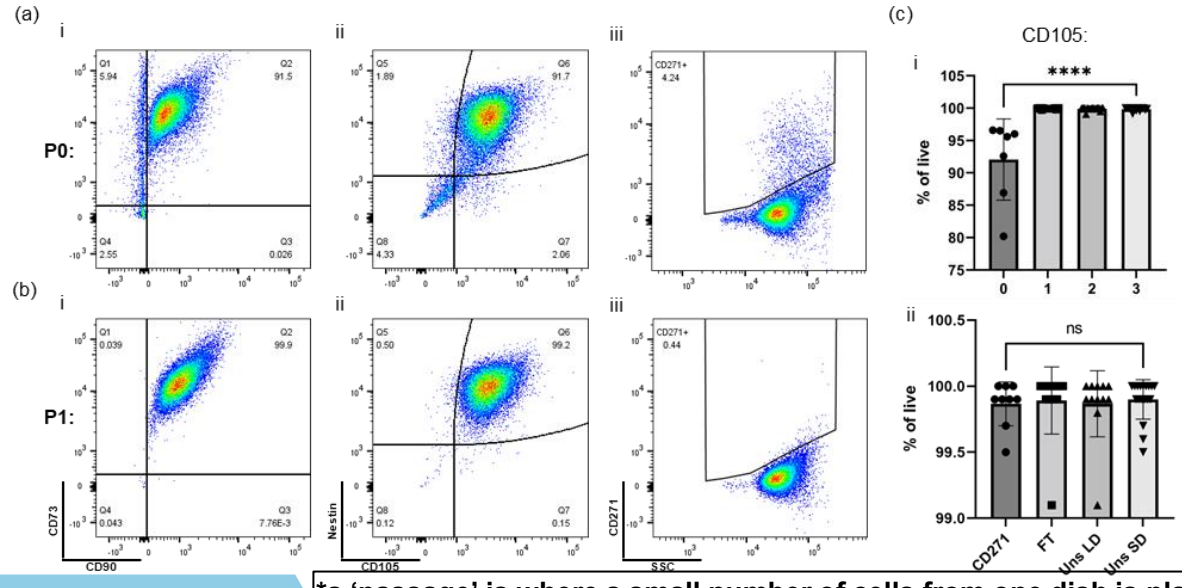

Figure 1*: Sorting of OM-MSCs is dispensable, with cells demonstrating an MSC profile within one passage.*
(a&b) FC was used to assess OM-MSCs at P0(a) and P1(b). CD73 & CD90(i), Nestin & CD105 (ii) and CD271 (iii) were evaluated.
(c) One marker was selected (CD105) to allow comparison of expression over several passages (i) or different sort conditions (ii). CD271= sorted by this marker, FT= flowthrough from the sort, Uns LD= unsorted cells started at low density, Uns SD= unsorted cells started at a standard density.

***a 'passage' is where a small number of cells from one dish is placed in a new one to expand**

The GMP compliant OM-MSCs could be induced into either fat (Oil Red O; Fig2.a) or bone (Alizarin Red S; Fig2.b), a key MSC characteristic.

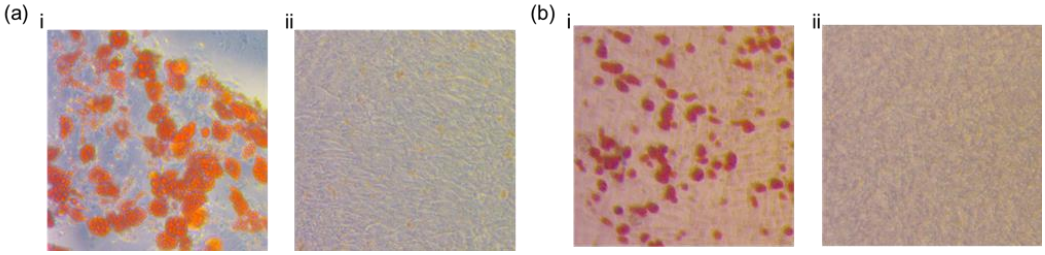

Figure 2: ***OM-MSCs derived by this method retain the characteristic MSC ability to become fat and bone cells.***
(a) Oil red O staining of lipid was evaluated on (i) differentiated and (ii) undifferentiated cells.
(b) Alizarin red S staining of calcium deposition was also evaluated on (i) differentiated and (ii) undifferentiated cells. Images were captured by light microscopy at 400x magnification.

The media OM-MSCs were grown in (termed 'Conditioned Media' or CM) was evaluated for inducing myelination in nerve cell placed in a dish. Myelination was compared between sorted and unsorted cells, to confirm that the unsorted GMP-compliant cells retained the pro-myelinating properties seen previously. Representative microscopy images (Fig3.a) are shown for unconditioned media (i, 'DMEM-'), CD271 sorted cells (ii), flowthrough cells from the sort (iii), unsorted cells initially grown at low density (iv), and unsorted cells grown at a standard density throughout (v). When made relative to the 'DMEM-' negative control (Fig3.b, dashed line at 1 represents control) we can see that CM from sorted and unsorted cells all induce myelination above control levels. We also see that there are no differences noted between the level of myelination obtained by either sorted or unsorted cells. ELISA measurement of CXCL12 levels in this CM (Fig3.c) confirms the presence of CXCL12, considered critical to this pro-myelinating effect. And, consistent with measurements of myelination, levels of CXCL12 were not significantly different between sorted and unsorted OM-MSCs.

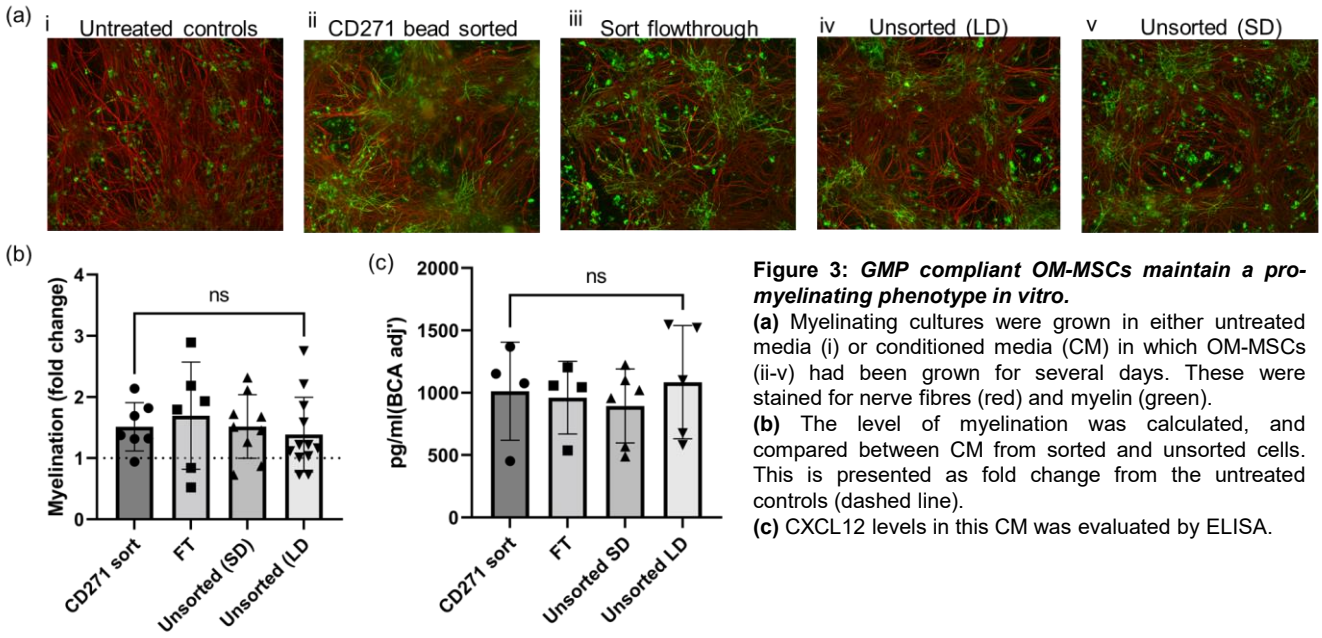

Figure 3: ***GMP compliant OM-MSCs maintain a pro-myelinating phenotype in vitro.***
(a) Myelinating cultures were grown in either untreated media (i) or conditioned media (CM) in which OM-MSCs (ii-v) had been grown for several days. These were stained for nerve fibres (red) and myelin (green).
(b) The level of myelination was calculated, and compared between CM from sorted and unsorted cells. This is presented as fold change from the untreated controls (dashed line).
(c) CXCL12 levels in this CM was evaluated by ELISA.

With the pro-myelinating properties of these cells confirmed, potential 'release criteria' can be set, These are defined standards that any future cell therapy product developed from them would have to meet before their clinical use. For OM-MSCs we propose that a FC profile of >95% CD73, CD90, CD105 and Nestin expression, as well as high levels of CXCL12 production, must be demonstratable. Additionally, for 3 of 3 samples tested in an NHS QC lab, endotoxin levels were below the limit of detection during the initial outgrowth phase without using antibiotics (lab reports available on request). Consequently, we would propose a similar 'below the limit of detection' cut-off for acceptable endotoxin levels in clinical usage, given their proposed use in the CNS.

Any potential cell therapy product are required to be stored and recovered from a deep-freeze, referred to as cryopreservation. This allows for samples of these cells to be 'banked' for future use, rather than requiring their fresh isolation from a new biopsy sample each time. However, it is necessary to confirm that cryopreserved cells (CP) maintain the same properties as their freshly isolated counterparts (Fresh). Consequently, OM-MSCs were recovered from cryopreservation, and evaluated by FC for MSC markers (Fig4.a), ELISA for CXCL12 (Fig4.b), and their ability to encourage myelination of nerve cells in culture (Fig4.c&d). These were compared to freshly isolated cells, with the properties of CP cells appearing unchanged by the cryopreservation and recovery process. This indicates OM-MSCs tolerate cryopreservation well, increasing their utility as a cell therapy product.

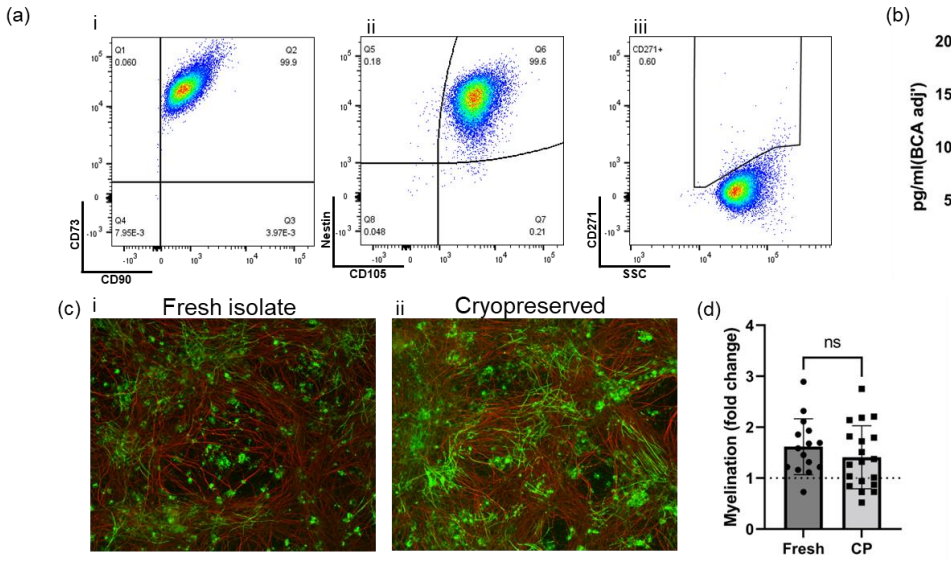

Figure 4: ***Cryopreservation of OM-MSCs doesn't effect their properties.*** **(a)** FC was used to assess CP OM-MSCs for CD73 & CD90(i), Nestin & CD105 (ii) and CD271 (iii) expression. **(b)** CXCL12 levels in CM from cryopreserved OM-MSC conditioned media was assessed and compared to fresh isolations of cells (Fresh). **(c)** Example microscopy images from myelinating cultures generated with CM produced from fresh and CP OM-MSCs are given, with axons in red and myelin in green. **(d)** As before, myelination was calculated relative to untreated controls (represented by the dotted line at 1), again comparing conditioned media from fresh and CP OM-MSCs.

Finally, the clinical effectiveness of these cells was assessed in the EAE mouse model. In EAE the immune system attacks myelin in the CNS, resulting in MS-like clinical symptoms, with mice scored on a scale of disease severity. As close as possible to a day after the initial onset of symptoms mice were injected with either GMP-compliant OM-MSCs (5% nLiven), non-GMP compliant OM-MSCs (10% FCS gelatin), or just the sterile fluid cells were suspended in (PBS). Their clinical scores (Fig5.a), and weight (Fig5.b) were assessed with the percentage survival also tracked (Fig5.c). The group of mice treated with GMP-compliant OM-MSCs demonstrated better recovery from disease (Fig5.a) compared with the untreated group. They also demonstrated better weight recovery (Fig5.b) and survival rates (Fig5.c) than untreated and non-GMP cell treated groups. However, it should be noted though that when subjected to statistical analysis these differences aren't considered statistically significant, possibly due to low numbers.

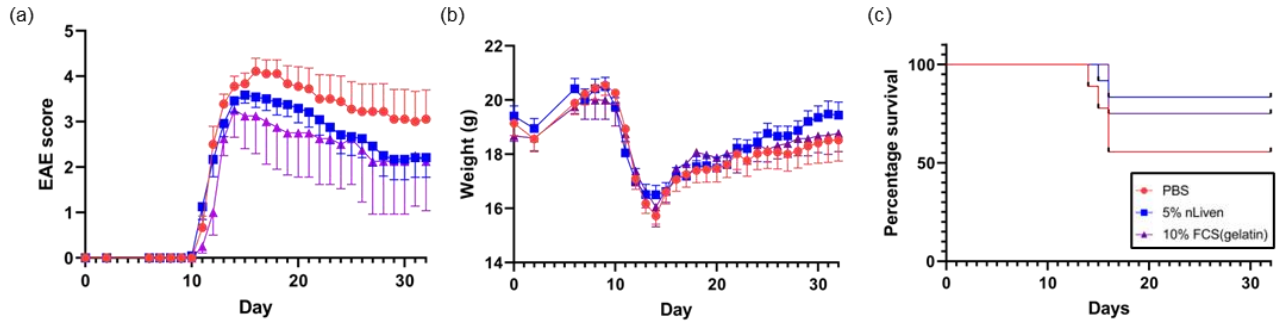

Figure 5: ***Animals treated with GMP compliant OM-MSCs demonstrate better clinical outcomes and improved survivability in the EAE model.*** EAE was induced in C57/BL6 mice and disease score **(a)** and weight **(b)** assessed daily for 32 days. One day after the onset of clinical symptoms (d13/14), mice were injected intravenously with either OM-MSCs grown in GMP compliant conditions (5% nLiven), OM-MSCs derived in non-GMP compliant conditions (10% FCS(gelatin)) or a vehicle control (PBS). **(c)** Survivability was assessed as a percentage of the original cohort surviving the full EAE course.

Overall, our findings tell us that OM-MSCs can be derived and expanded in GMP-compliant conditions, and that a homogenous population can be obtained using just culture methods without sorting. The resulting OM-MSCs have all the required markers of MSC identity, while retaining the pro-myelinating properties seen previously in culture. In an animal model of MS their administration demonstrates a trend toward better disease outcomes, although their evaluation in more pre-clinical models would be prudent.

WHAT IMPACT COULD THE FINDINGS HAVE?

The fact that we were able to grow OM-MSCs from all human nasal tissue samples received with typical MSC properties and in conditions that would allow the cells to be given to patients supports their validation for future grant application for a clinical trial. OM-MSCs potentially could have a use for the treatment of multiple sclerosis or other demyelination disorders. We have already shown lab grown OM-MScs can promote repair in various assays.

HOW WILL THE OUTCOMES BE DISSEMINATED?

We are currently in the process of preparing a manuscript for publication, with our intention being to submit to Cytotherapy, a respected journal in the field.

As this is a collaborative project with the Scottish National Blood Transfusion Service (SNBTS), we have also been sharing our findings with them throughout, in particular with member of their Tissues, Cells and Advanced Therapeutics (TCAT) division.

The work could also be presented at future international scientific conferences.

CONCLUSION

The findings presented in this study demonstrate that, using our new isolation and culture protocols, sterile, GMP compliant OM-MSCs could be easily generated from human nasal biopsy samples (a readily accessible site for sampling) taken from multiple donors. These cells can be identified as MSCs by reproducible methods and have been demonstrated to promote myelination in a dish and a model of MS. They can also be frozen, stored and recovered without issue. Given this, we propose OM-MSCs generated by this method may have considerable utility as a cell therapy product in CNS injury and disease. This could be on both an autologous basis as freshly derived cells, and potentially as a bankable cell product.

RESEARCH TEAM & CONTACT

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Additional Information

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