Electronic Supplementary Information for

Magnetic field application or mechanical stimulation via magnetic microparticles does not enhance chondrogenesis in mesenchymal stem cell sheets

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Modeling of forces on a magnetic particle

Force on a magnetic particle due to the magnetic field

Force exerted on the magnetic Dynabeads M-450 particles (MP) by the N52 magnets was calculated with equation [1]

$$F_m = V_m (M \cdot \nabla) B \qquad \textbf{[1]}$$

where F_m is the force experienced, V_m is the volume of the MP, M is the volumetric magnetization, and $(M \cdot \nabla)B$ is the magnetic field gradient in the direction of particle magnetization [1]. Assuming that the moment of each magnetic particle aligns with the direction of the magnetic field, equation [1] is simplified to

$$F_m = V_m M \nabla B \qquad \textbf{[2]}$$

where ∇B is the magnetic field gradient of the local field magnitude B.

The MP have a diameter of 4.40 µm [2], thus the volume (V_m) of the MP is 4.46 x 10^{-17} m^3 .

Volumetric magnetization (M) is the product of the mass magnetization (σ) at a given magnetic field and the density of the MP (ρ).

$$M = \sigma \rho \qquad \textbf{[3]}$$

The density of the MP (ρ) is 1600 kg/m^3 [3]. Mass magnetization (σ) of a MP was interpolated from the magnetization curve of Dynabeads M-450 (Fig S.1) [2]. The magnetic field was calculated through modeling of the experimental set-up with Finite Element Method Magnetics (FEMM 4.2). The FEMM default properties of N52 magnets were used. For VMF groups, Aluminum 6061-T6, which does not affect the magnetic field, was selected to model the rotating agitator axle to which the 30 individual 1.27 cm cube magnets were affixed. Closest (3.29 mm) and farthest (29.72 mm) positions of the magnet from the cell culture insert along the path of rotation in the axial plane were used to calculate the maximum and minimum magnetic field on tissue sheets during VMF stimulation. In the static magnetic field group (SMF), the magnetic field was taken at the permanent distance (2.28 mm) between the tissue sheets and the single 5.08 cm x 5.08 cm x 1.27 cm (LxWxH) magnet.

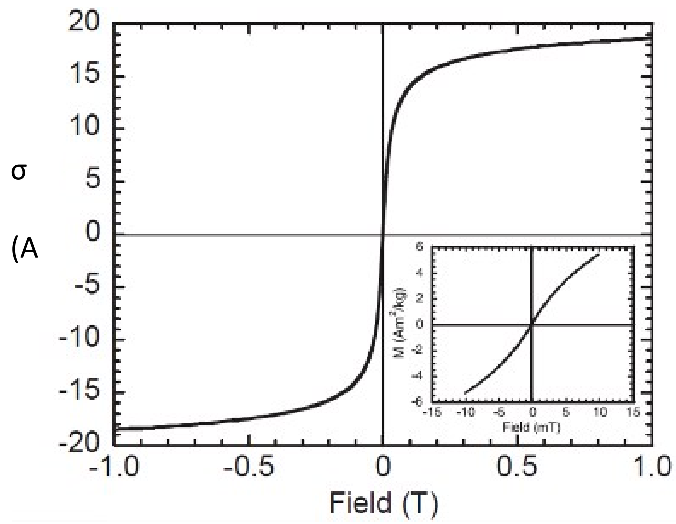

Fig S.1. Magnetization hysteresis loop (mass magnetization (***σ***) vs magnetic field) of Dynabeads M-450 characterized by and adapted with permission from Fonnum et al. [2].

Magnetic gradient (∇B) was calculated with the data produced by FEMM by dividing the difference in magnetic field by the distance between the respective finite elements. As the largest magnetic field gradient was along the path toward the closest point on the magnet, the y direction in the FEMM model, the magnetic field gradient was simplified to $\nabla B = \dfrac{\partial B}{\partial y}$, which represents the change in magnetic field in the y direction. The small magnetic field gradients in the z and x directions, which would slightly increase the total force on the MP, were not considered in the maximum and minimum force calculations.

Maximum force on a magnetic particle due to the variable magnetic field

Maximum magnetic field strength and gradient were achieved by minimizing the distance from the corner of the magnet to the cells to 3.29 mm through optimization of the experimental set-up (Fig S.2) in the VMF groups. At this position, the magnetic field strength calculated by FEMM is $2.84x10^{-1}$ T. The corresponding σ value from Fig S.1 to this field strength is $1.66x10^{1}$ Am^2/kg. Therefore, volumetric magnetization from equation [3] is

$$M = 1.66x10^{1}\frac{Am^2}{kg} * 1600\frac{kg}{m^3} = 2.65x10^{4}\,\frac{A}{m}$$

∇B calculated from the data produced by FEMM is $5.37x10^{1}$ T/m = $5.37x10^{1}$ N/Am^2. Using equation [2], the force is

$$F = 4.46x10^{-17}m^3 * 2.65x10^{4}\frac{A}{m} * 5.37x10^{1}\frac{N}{Am^2} = 6.36x10^{-11}\,N$$

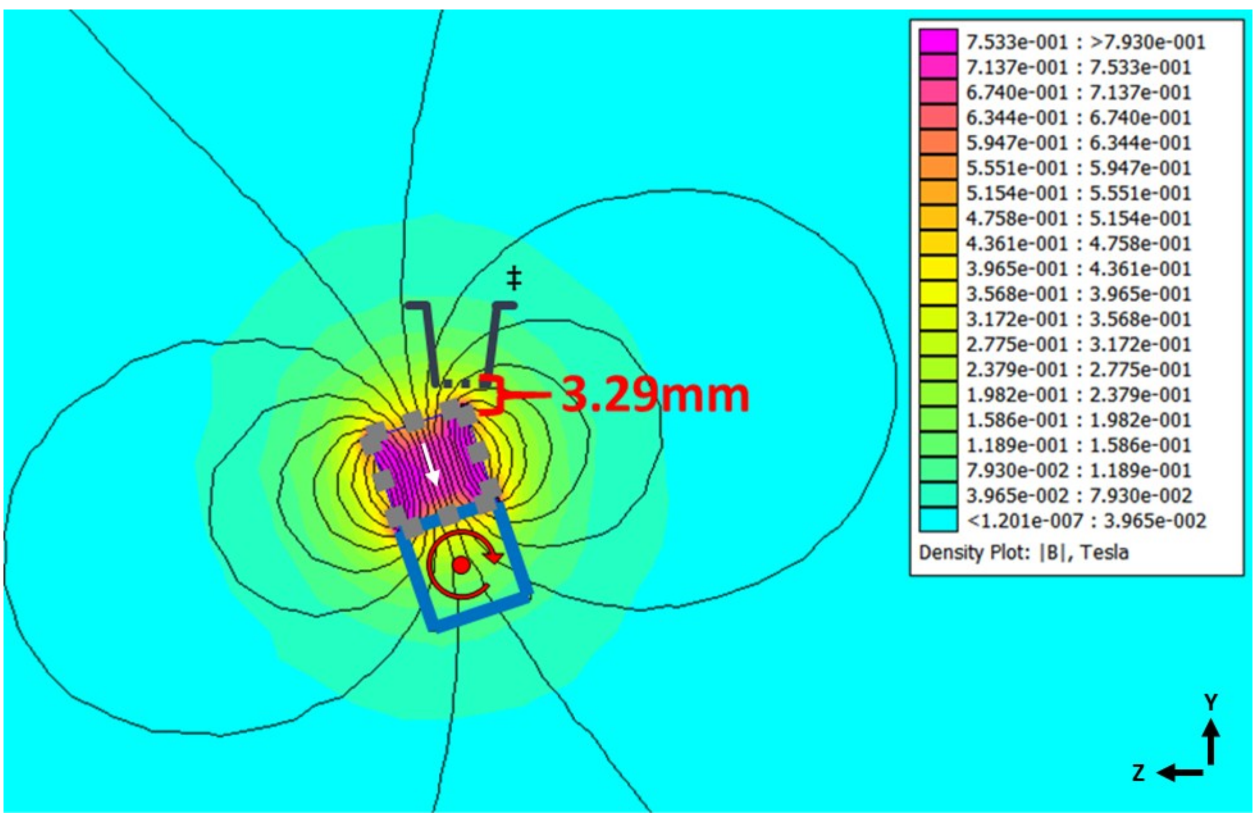

Fig S.2. Magnetic field density plot in the axial plane for maximum force configuration. Field density decreases from pink to blue. The solid blue line denotes the aluminum bar that the magnets are attached to and the gray dotted line denotes the magnet. The white arrow points to the north pole of the magnet. The red arrow and dot denote path of rotation. The structure denoted by ‡ represents the cell culture insert.

Thus, 63.6 pN of force is imparted upon the MP by the magnets in the maximum force configuration during VMF stimulation.

Minimum force on a magnetic particle due to the variable magnetic field

Minimum magnetic field strength and gradient in VMF groups were achieved by maximizing the distance from the surface of the magnet to the cells to 29.72 mm (Fig S.3). At this position, the magnetic field strength calculated by FEMM is $3.03x10^{-2}$ T in the negative y direction. The corresponding σ value from Fig S.1 to this field strength is -9.66 Am^2/kg. Therefore from equation [3], the volumetric magnetization is

$$M =- 9.66\frac{Am^2}{kg} * 1600\frac{kg}{m^3} =- 1.55x10^4\,\frac{A}{m}$$

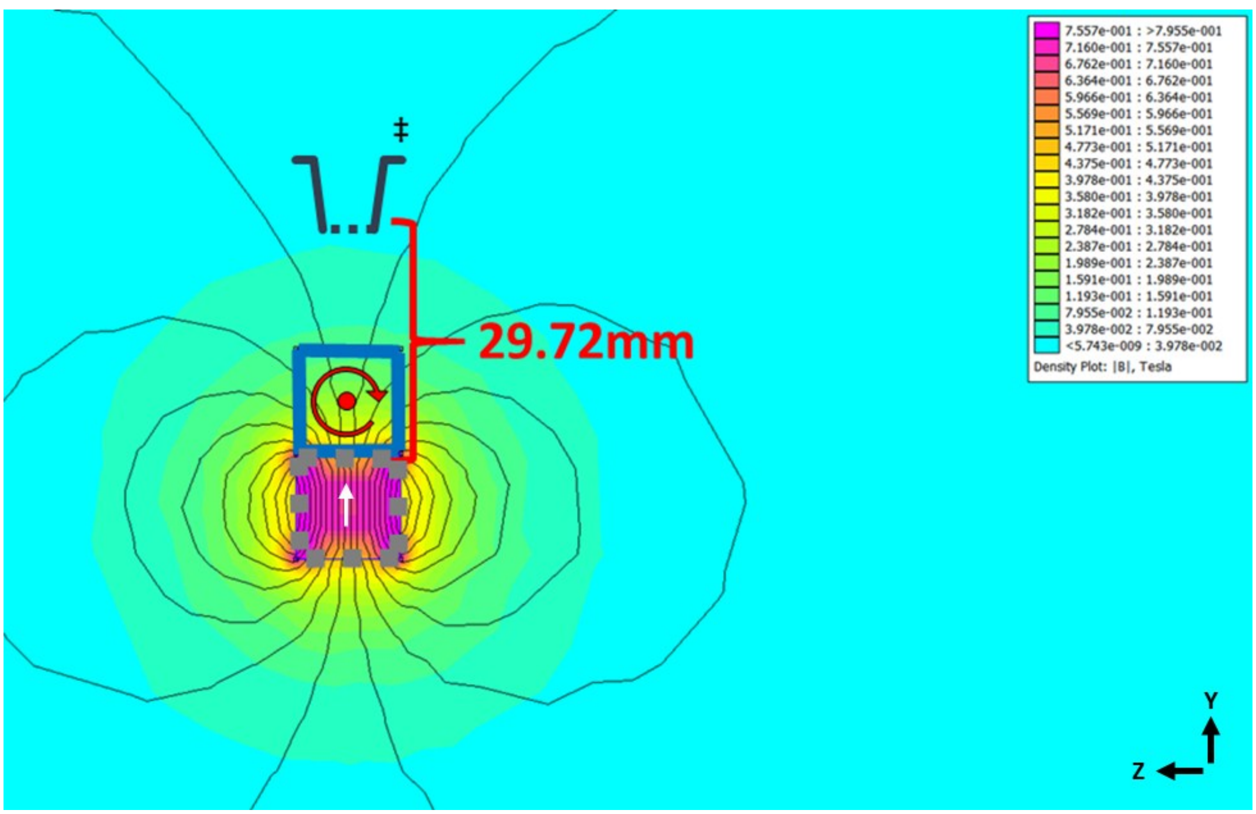

Fig S.3. Magnetic field density plot in the axial plane for minimum force configuration. Field density decreases from pink to blue. The solid blue line denotes the aluminum bar that the magnets are attached to and the gray dotted line denotes the magnet. The white arrow points to the north pole of the magnet. The red arrow and dot denote center of rotation. The structure denoted by ‡ represents the cell culture insert.

∇B calculated from the data produced by FEMM is -1.54 T/m = -1.54 N/Am^2. Using equation [2], force on each MP is

$$F = 4.46x10^{-17}m^3 * -1.55x10^4\frac{A}{m} * -1.54\frac{N}{Am^2} = 1.06x10^{-12}\,N$$

Thus, 1.06 pN of force in the negative y direction is imparted upon the MP by the magnets in minimum force configuration from the axial plane during VMF stimulation.

Calculation of minimum force in the longitudinal plane (Fig S.4) confirmed that the magnitude of the force calculated in the axial plane is larger, thus representative of the minimum force experienced in any plane. The data from the same distance of 29.72 mm was used in calculations. At this position, the magnetic field strength calculated by FEMM is -1.21x10^{-3} T. The corresponding σ value from Fig S.1 to this field strength is -1.81 Am^2/kg. Therefore from equation [3],

$$M = -1.81\frac{Am^2}{kg} * 1600\frac{kg}{m^3} = -2.90x10^3\,\frac{A}{m}$$

∇B calculated from the data produced by FEMM is -1.11x10^{-1} T/m = -1.11x10^{-1} N/Am^2. Using equation [2],

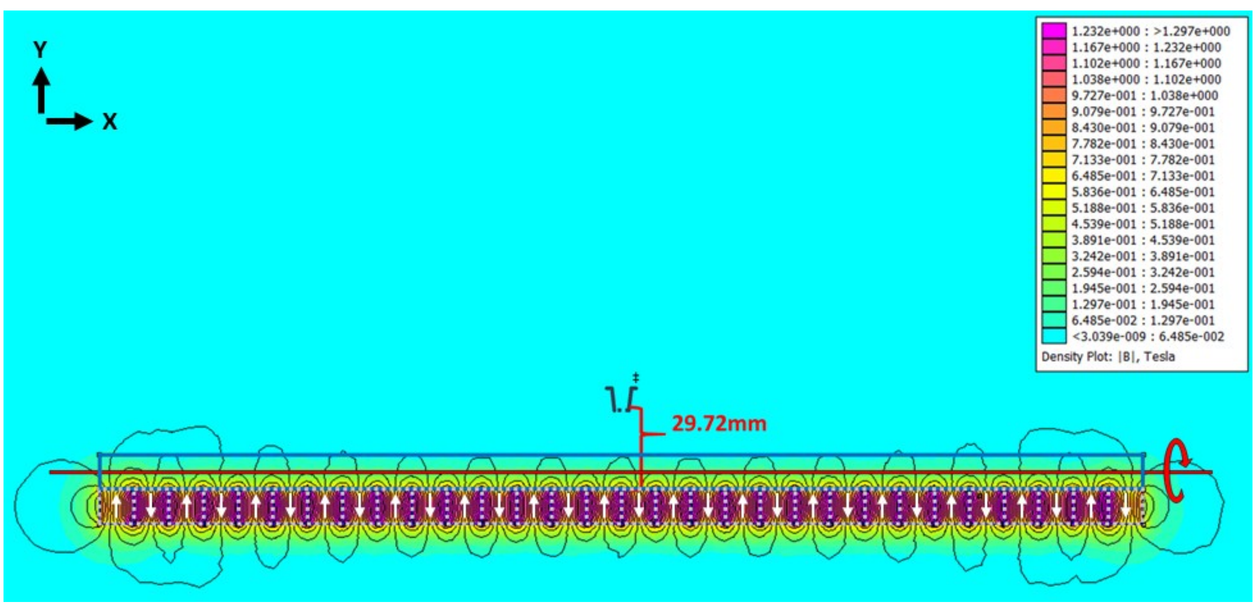

Fig S.4. Magnetic field density plot in the longitudinal plane for minimum force configuration. Field density decreases from pink to blue. The solid blue line denotes the aluminum bar that the magnets are attached to and the dotted gray lines denote the magnets affixed in alternating pole orientation (white arrows point to the north poles of the magnets). The red arrow and line denote path of rotation. The structure denoted by ‡ represents the cell culture insert.

$$F = 4.46x10^{-17} m^3 * -2.90x10^3 \frac{A}{m} * -1.11x10^{-1} \frac{N}{Am^2} = 1.43x10^{-14}\, N$$

Thus, 0.0143 pN of force in the negative y direction is imparted upon the MP by the magnets in minimum force configuration from the longitudinal plane during VMF stimulation.

Because FEMM is a 2-D simulator, the y direction forces on a MP are not the same in axial and longitudinal planes. FEMM cannot reconcile magnetic gradients calculated at one point in space from different perspectives.

Constant force on a magnetic particle due to the static magnetic field

Sheets in the SMF group, positioned 2.28 mm from the single magnet, experienced a constant magnetic field strength and gradient. At this position, the magnetic field strength calculated by FEMM is $1.80x10^{-1}$ T. The corresponding σ value from Fig S.1 to this field strength is $1.56x10^{1}$ Am^2/kg. Therefore from equation [3], the volumetric magnetization is

$$M = 1.56x10^1 \frac{Am^2}{kg} * 1600 \frac{kg}{m^3} = 2.50x10^4 \frac{A}{m}$$

∇B calculated from the data produced by FEMM is 3.94 T/m = 3.94 N/Am^2. Using equation [2], force on each MP is

$$F = 4.46x10^{-17} m^3 * 2.50x10^4 \frac{A}{m} * 3.94 \frac{N}{Am^2} = 4.39x10^{-12}\, N$$

Thus, 4.39 pN of force is imparted upon the MP by the magnets during SMF stimulation.

Data from supplementary experiments of hMSC sheets without and with magnetic particles stimulated by magnetic fields

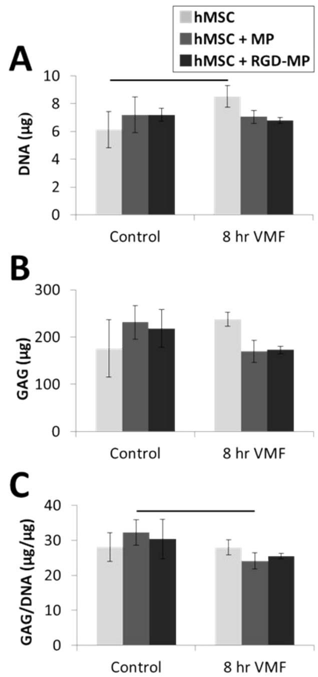

Fig S.5. (A) DNA, (B) GAG and (C) GAG normalized to DNA of hMSC sheets without magnetic particles (MP) (light gray), hMSCs with serum-coated MP (dark gray) as described in the methods and hMSCs with RGD-coupled MP (black) from donor 1 passage 2. RGD was coupled to MP as previously described [4]. Control sheets were never exposed to a magnetic field. Starting on day 4, stimulated sheets were exposed to a variable magnetic field (VMF, 0.95 cm diameter x 1.27 cm height cylindrical magnets, 0.39 T, CMS Magnetics) for 8 hours daily 7 days/week for 3 weeks. Lines denote statistical significance of $p<0.05$.

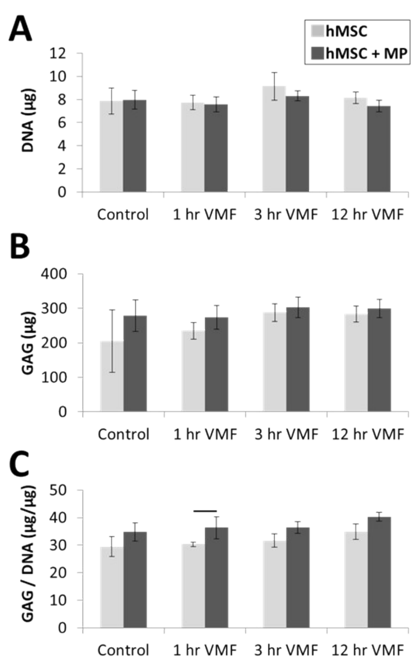

Fig S.6. (A) DNA, (B) GAG and (C) GAG normalized to DNA of hMSC sheets with (dark gray) and without (light gray) magnetic particles (MP) from donor 1 passage 2. Control sheets were never exposed to a magnetic field. Starting on day 1, stimulated sheets were exposed to a variable magnetic field (VMF, 1.27 cm cube magnets, 1.45 T, CMS Magnetics) for 1 hour or 3 hours daily 6 days/week or for 12 hours daily 7 days/week for 3 weeks. Lines denote statistical significance of $p<0.05$.

References

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3. J. Xu, K. Mahajan, W. Xue, J. O. Winter, M. Zborowski and J. J. Chalmers, *J Magn Magn Mater*, 2012, **324**, 4189-4199.
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