Ultrasound-Assisted Manipulation of Micro-particles in Fluid Matrix to Create Highly Aligned Constructs

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1. INTRODUCTION

- Structural anisotropy, often observed in composite materials such as wood and in human tissues, is central to the function of these materials.
- For example, Balsa wood is characterized by densely packed honeycomb arrangement of cells-fibers that results in its high strength to weight ratio.
- Similarly, cells and collagen fibers of ligaments/tendons are aligned along the direction of tensile loading which these tissues typically experience.

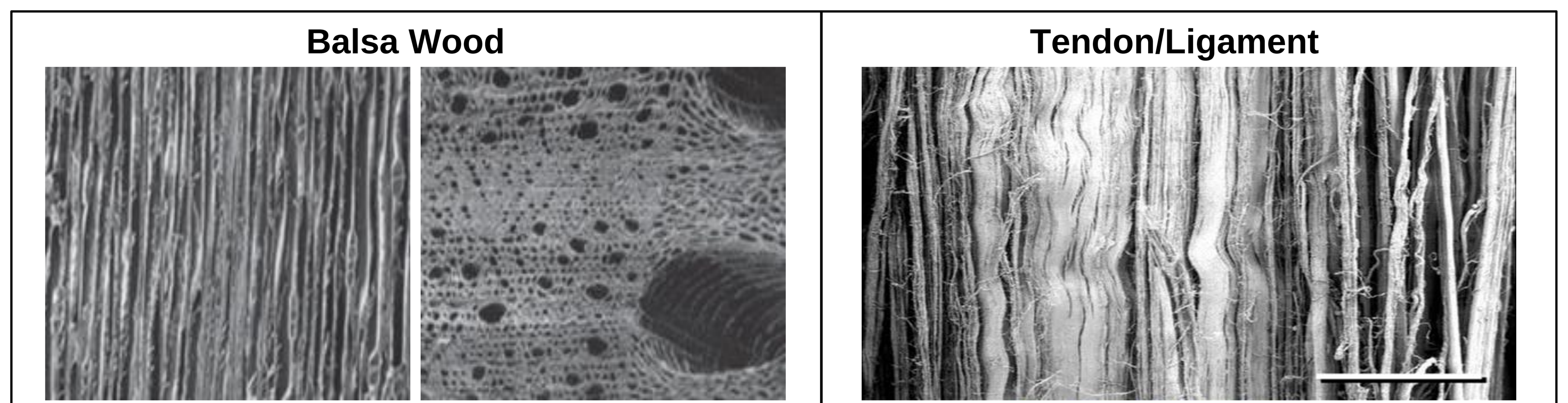

Fig. 1: Microscopic images depicting the organization of different natural composites[1,2]

- When engineering composites and tissues, it is important to recapitulate the organization of micro-constituents such as cells, particles, and fibers in order to mimic the characteristics of the original materials.
- Here, we study the use of ultrasound to create bulk standing pressure waves in fluid matrices as a non-contact approach to preferentially organize suspended micro-constituents in 3D.

Primary Objectives of this study:

1. Investigate the system design and effect of critical process parameters (ultrasound frequency and amplitude) on the alignment of micro-particles in fluid matrices.
2. Demonstrate proof-of-concept for patterning living human cells within a biocompatible hydrogel.

2. MATERIALS AND METHODS

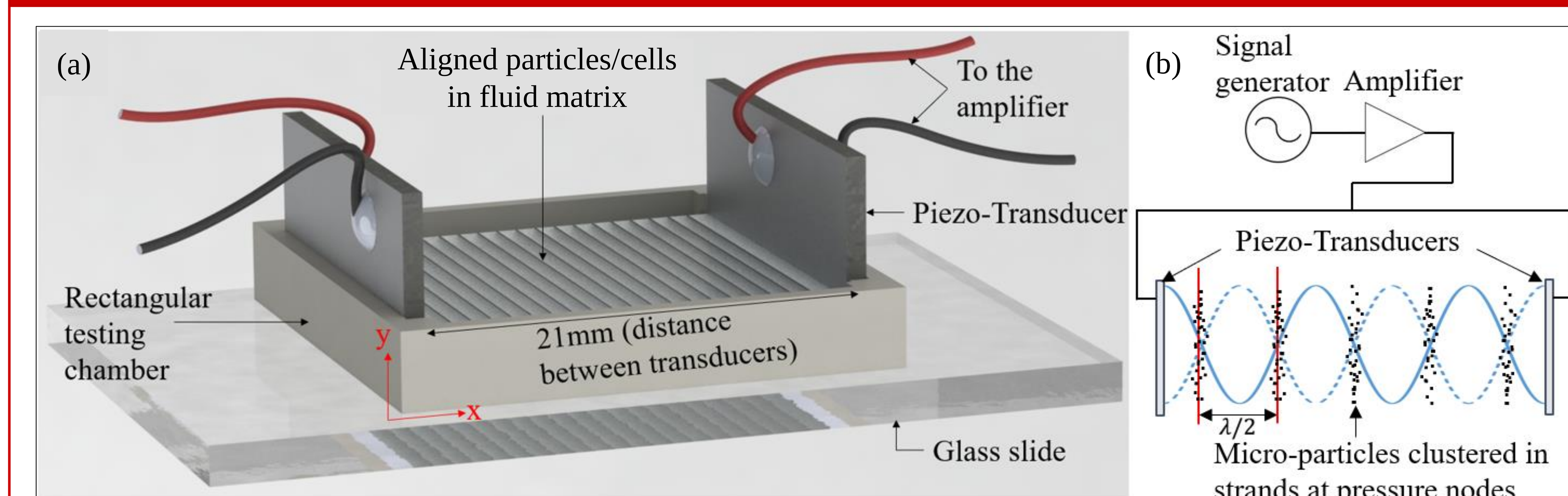

Fig. 2: (a) Schematic of ultrasound-assisted manipulation testing system design; (b) Basic circuitry to drive the transducer setup in parallel at a particular frequency and voltage amplitude.

- Pressure waves in fluid caused by the vibration of opposite transducers interfere to produce a standing bulk acoustic pressure wave[3]: $p(x,t) = P_o \mathrm{Cos}(\omega t)\mathrm{Cos}(kx)$, where ω is the angular frequency, k is the wavenumber and P_0 is the pressure amplitude close to the transducer.
- The nodes of standing wave are parallel to the walls of the transducers and are separated by λ/2.
- The suspended micro-particles are pushed to the nodes due to the acoustic radiation forces[4]:
  $F_{rad} = (\pi/3)(k_l - k_p) r^3 (2\pi/\lambda) P_o^2 \mathrm{Sin}(4\pi x/\lambda)$, where k_p and k_l are the compressibility of the particle and fluid respectively, and λ is the wavelength of ultrasound.

Study1: Manipulating polymer particles in water (Fig 3a):

Suspension	Polyethylene (PE) micro-spheres (mean Ø 8 µm) suspended in water at 2 mg/mL
Process parameters	Frequency: 1, 1.5, 2, 3 MHz Voltage amplitude: 80 mVpp, 160 mVpp
Metric	Inter-strand spacing and alignment time
Method	Optical microscopy and ImageJ analysis

Study 2: Manipulating MG63 cells in hydrogel (Fig 3b):

Suspension	Neutral red stained MG63 cells (mean size 15 µm) suspended in Alginate at 2 mg/mL
Process parameters	Frequency: 2 MHz Voltage amplitude: 160 mVpp
Evaluation	Inter-strand spacing and cell viability
Method	Fluoroscence microscopy at 590 nm

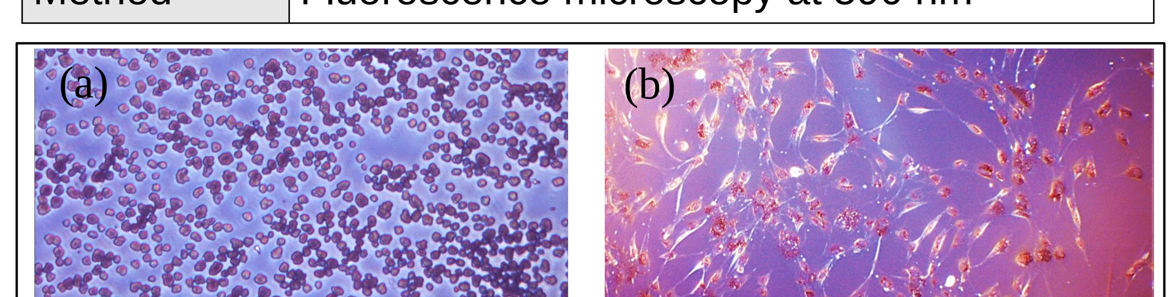

Fig. 3: a) PE micro-particles; b) Neutral red stained MG63 cells.

3. RESULTS AND DISCUSSION

Study 1: Alignment of Polyethylene micro-particles in water:

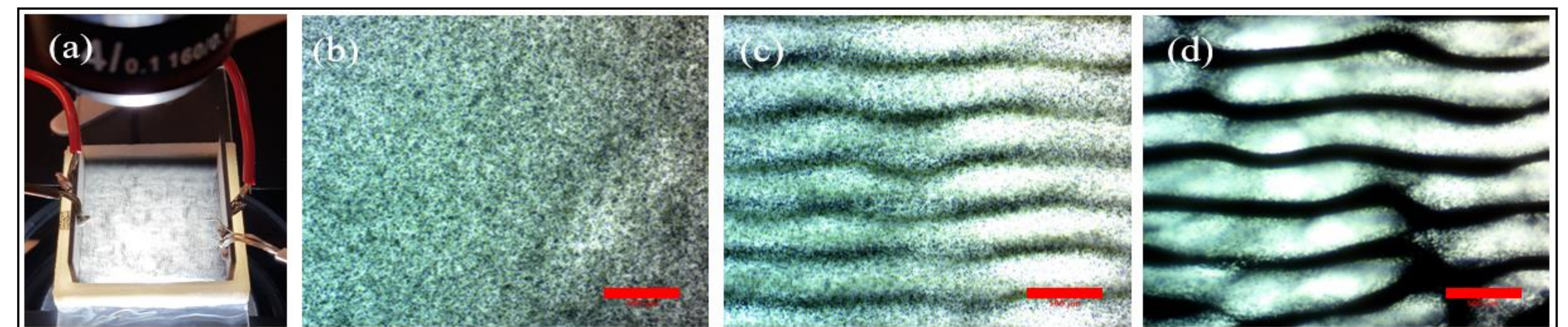

Fig. 4: (a) Experimental setup with 3 MHz transducers; (b-d) Organization of micro-particles at time points 0, 30 and 60 s respectively. Magnification = 4X; Scale bar = 500 µm

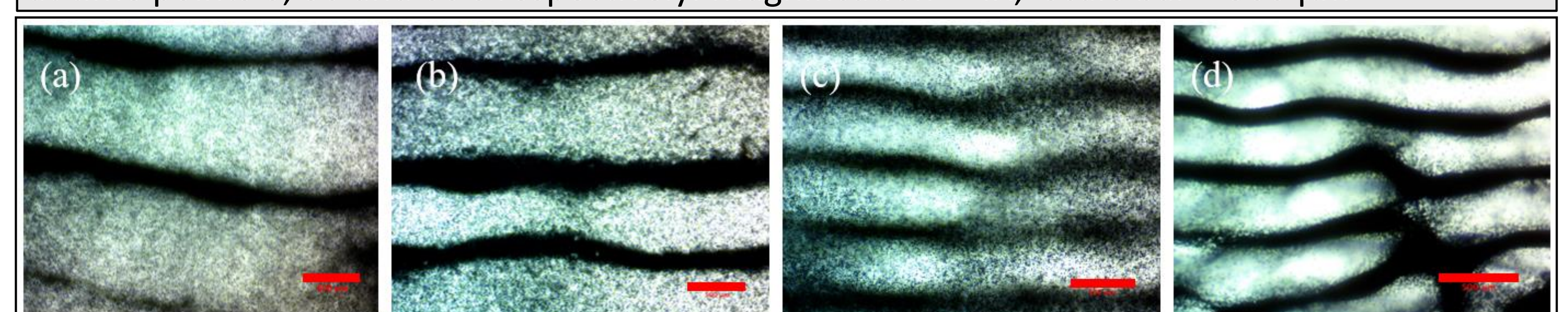

Fig. 5: Alignment of micro-particles after 60 s at 80 mVpp for (a) 1 MHz, (b) 1.5 MHz, (c) 2 MHz, and (d) 3 MHz. Scale bar = 500 µm

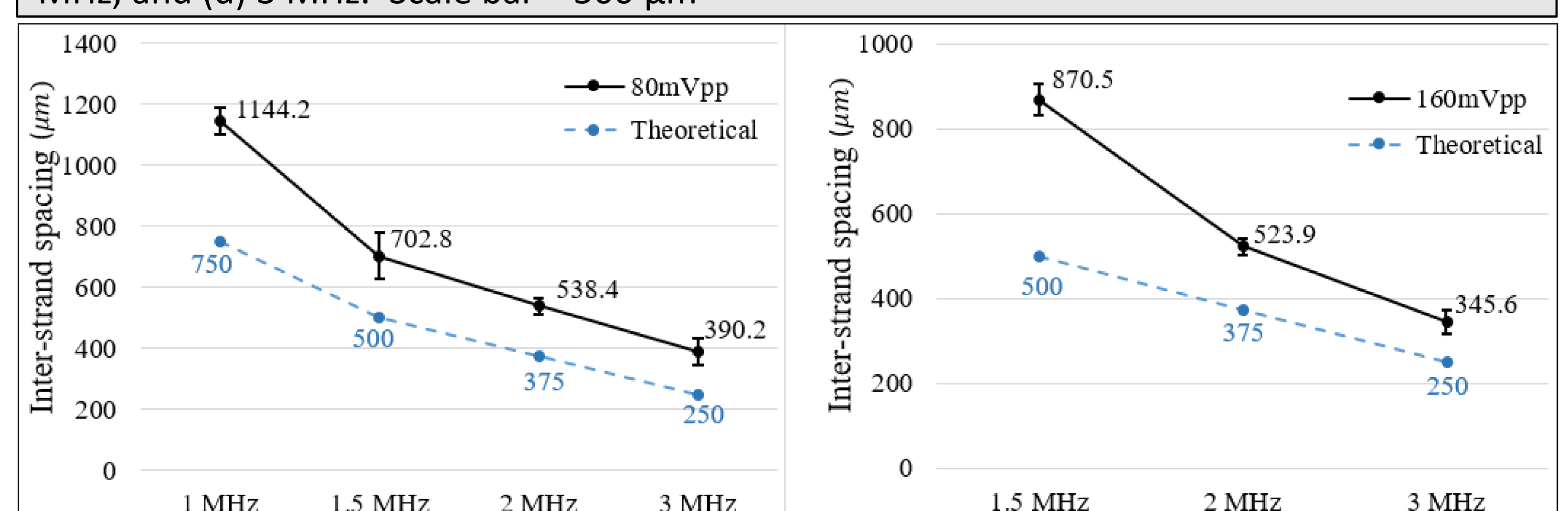

Fig. 6: Observed and theoretical inter-strand spacings between aligned micro-particles. Spacing was inversely proportional to the frequency ($p < 0.05$), but was larger than the theoretical estimate. Spacing at 160 mVpp were not significantly different from 80 mVpp of excitation voltage ($p > 0.05$)

Study 2: Alignment of MG63 cells in Alginate hydrogel at 2 MHz frequency:

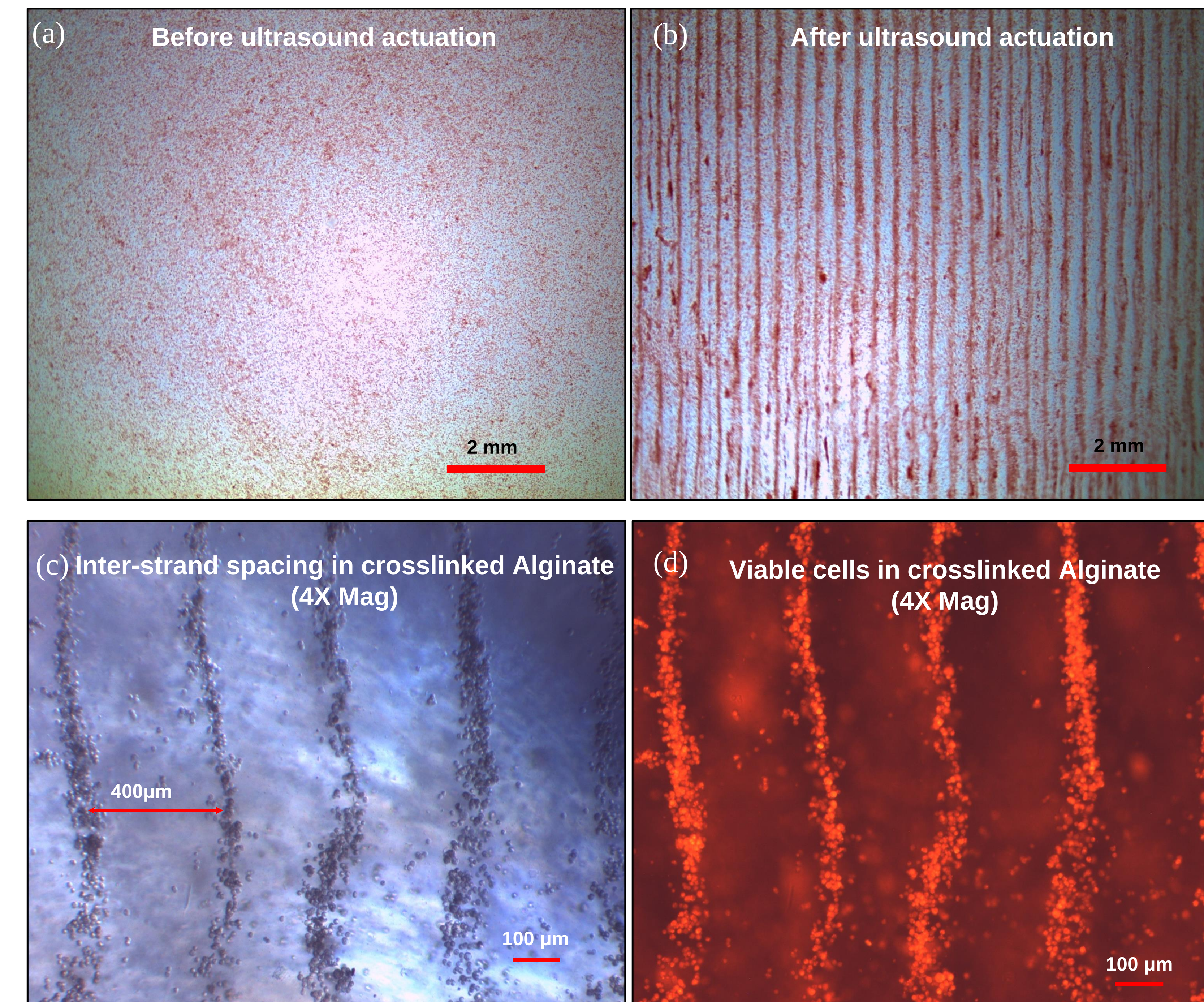

Figure 7: (a) Neutral red-stained MG63 cells suspended in alginate prior to ultrasound actuation; (b) Cells aligned using 2 MHz frequency; (c) 4X Magnified optical image of cells post-alignment and crosslinking; (d) Fluorescence image of cells (neutral red uptake visible in living cells at 590 nm). Cells were 100% viable post-alignment.

4. CONCLUSION AND FUTURE SCOPE

- Both polymer micro-particles (in water) and MG63 cells (in Alginate hydrogel) could be aligned in linear patterns within 60 s of ultrasound actuation.
- Inter-strand spacing was inversely proportional ultrasound frequency ($p < 0.05$), but the voltage amplitude did not have a significant effect.
- Ultrasound (2 MHz) was not detrimental to living cells.
- Future studies would entail optimizing the setup design and investigating the fabrication of functional constructs with various alignment patterns.

5. REFERENCES

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6. ACKNOWLEDGEMENTS

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