Supporting Information

Collagenase Motors in Gelatine-Based Hydrogels

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Table S1. Overview of the silica particle properties

Silica Particle	Mass (mg)[a)]	Surface area (μm²)[a)]	Diameter (μm)[a)]
$^{0.5}$M	1×10^{-10}	0.8	0.5
$^{0.8}$M	6×10^{-10}	2.0	0.8
^{1}M	1×10^{-9}	3.1	1.0
^{4}M	9×10^{-9}	12.6	2.0
^{R}M	2×10^{-10}	1.4	1.5 (width) 0.3 (length) 5.6 (aspect ratio)

[a)] of a single particle

Table S2. Overview of the collagenase immobilization characteristics

Motor	Silica particle stock solution (mg mL^{-1})	Particle V (polymer brush coated) (μL)[a)]	Mass of Coll for immobilization (mg)[b)]	Total mass of Coll/Total area of motor (μg μm^{-2})[c)]
^{R}M^{Coll}			1.5	4×10^{-8}
$^{0.5}$M^{Coll}	30	66	1.4	5×10^{-8}
$^{0.8}$M^{Coll}	50	60	1.6	9×10^{-8}
^{1}M^{Coll}	50	80	3.2	3×10^{-8}
^{2}M^{Coll}	50	180	2.4	8×10^{-8}
$^{0.5}$M$^{Coll(low)}$	30	45	0.4	NA[d)]

[a)]particle volume used for Coll coating to have comparable total surface area of 1×10^{10} μm²; [b)]from a 4 mg ml^{-1} Coll stock solution in HEPES buffer;[c)]experimentally determined from a BCA analysis (n=2); [d)]below the detection limit of the BCA analysis.

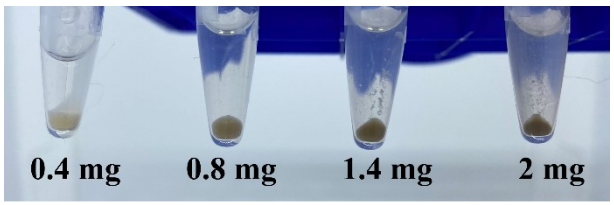

Figure S1. Photo of $^{0.5}M^{Coll}$ after being coated with different amounts of collagenase. $^{0.5}M^{Coll(low)}$ and $^{0.5}M^{Coll}$ correspond to the frist tube (0.4 mg collagenase) and third tube (1.4 mg collagenase).

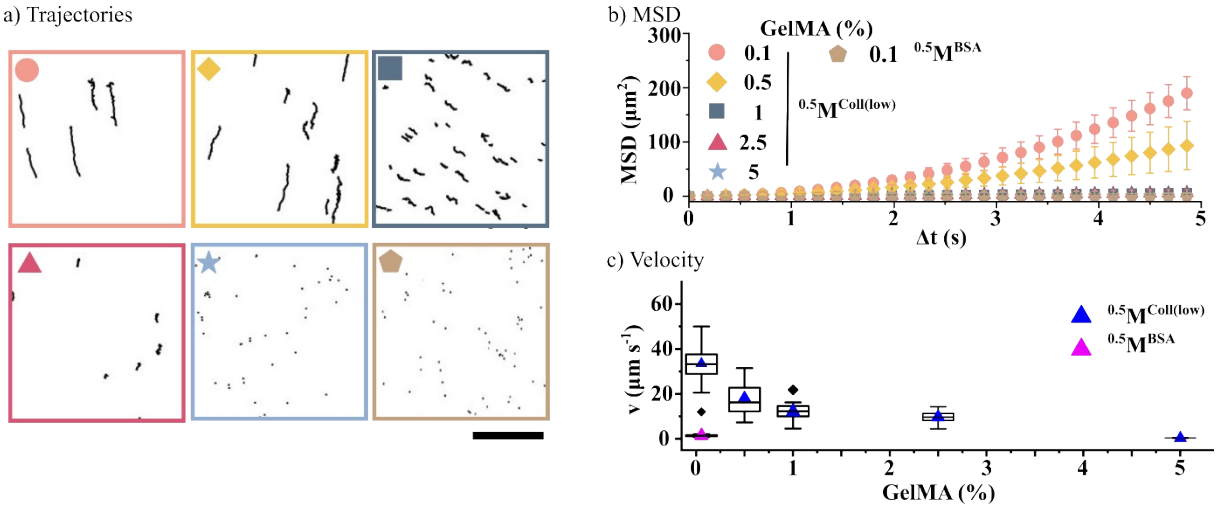

Figure S2. Locomotion properties of $^{0.5}M^{Coll(low)}$. Trajectories maps (a) and MSD plots (b) of $^{0.5}M^{Coll(low)}$ moving in different viscosity gelatine-based environments when triggered with 2 mM calcium. Scale bar is 50 µm. c) Velocities of $^{0.5}M^{Coll(low)}$ represented as whisker plots. $^{0.5}M^{BSA}$ is used as control.

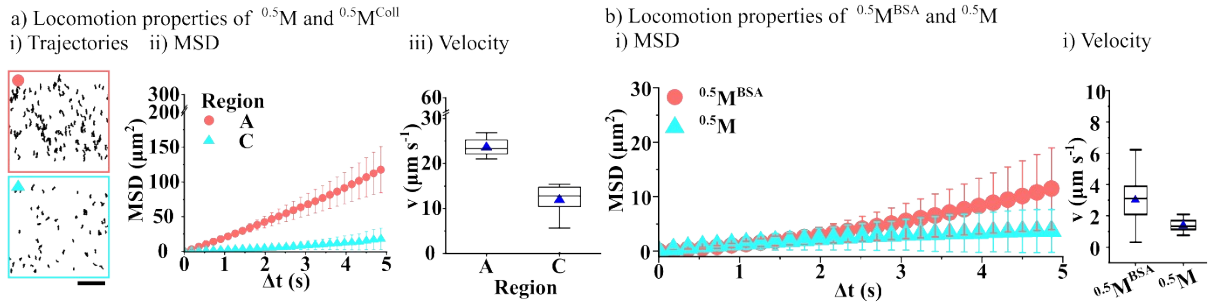

Figure S3. a) Mixture of motors $^{0.5}M$ and $^{0.5}M^{Coll}$ in the regions A (close to the inlet) and C (at the other end of the microfluidic channel). Trajectories maps (i) and MSD plots (ii) of $^{0.5}M^{Coll}$ and $^{0.5}M$ moving in an environment made of 1% GelMA when triggered with 2 mM calcium. Scale bar is 100 µm. iii) Velocities of $^{0.5}M^{Coll}$ and $^{0.5}M$ represented as whisker plots. b) i) MSD plots of $^{0.5}M^{BSA}$ and $^{0.5}M$ in 1 % GelMA using 2 mM calcium as the trigger (not as a mixed population). ii) Velocities of $^{0.5}M^{BSA}$ and $^{0.5}M$ represented as whisker plots.

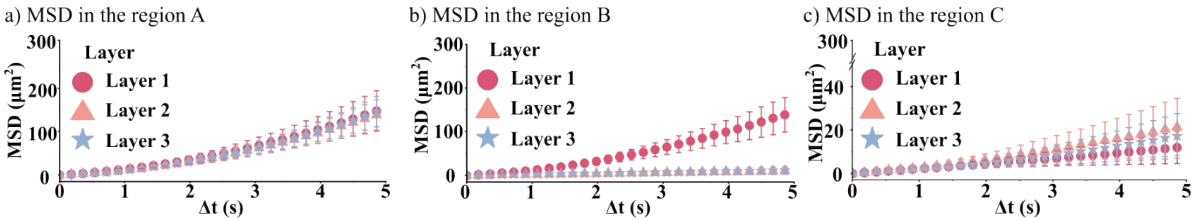

Figure S4. MSD plots of $^{0.5}M^{Coll}$ in the region A (a), the region B (b) and the region C (c) in 1 % GelMA using 2 mM calcium as the trigger.

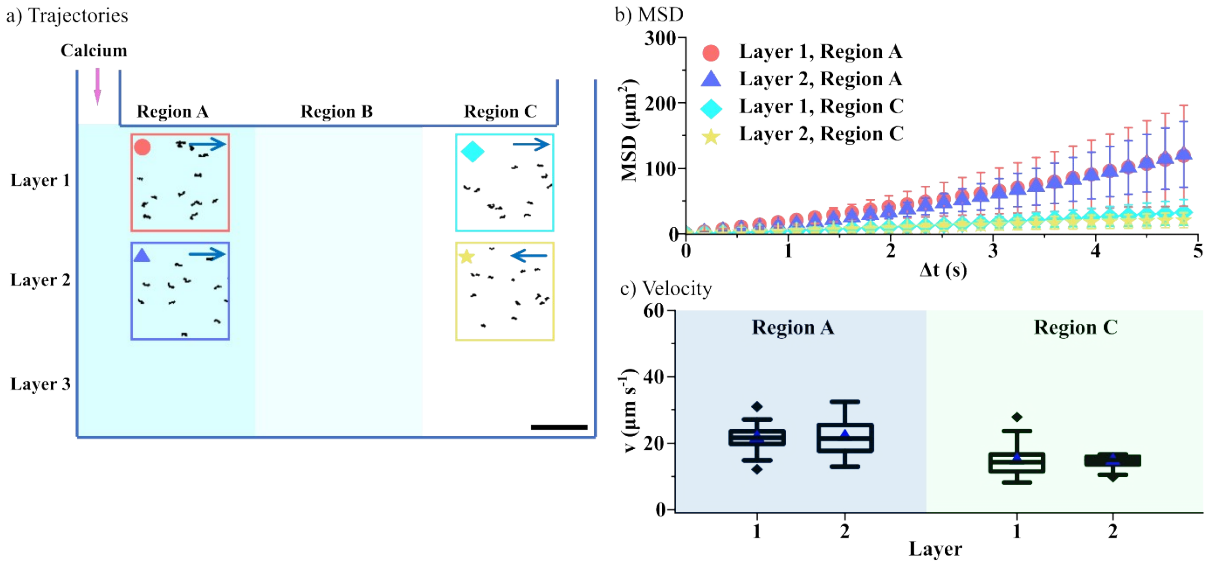

Figure S5. Motors $^{0.5}M$ and $^{0.5}M^{Coll}$ in different positions. Trajectories maps (a) and MSD plots (b) of $^{0.5}M^{Coll}$ and $^{0.5}M$ moving in an environment made of 1% GelMA when triggered with 2 mM calcium. Scale bar is 50 µm. c) Velocities of $^{0.5}M^{Coll}$ and $^{0.5}M$ represented as whisker plots.

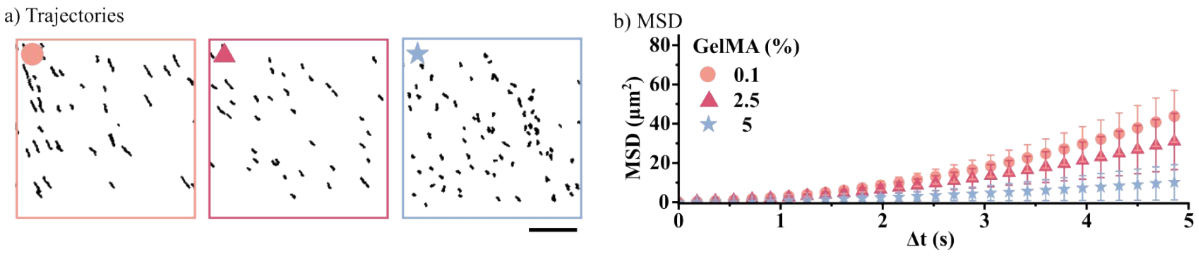

Figure S6. Trajectory maps (a) and MSD plots (b) of $^{0.8}M^{Coll}$ in hydrogels made from 0.1, 2.5, or 5 % GelMA using 2 mM calcium as the trigger. Scale bar is 100 µm.

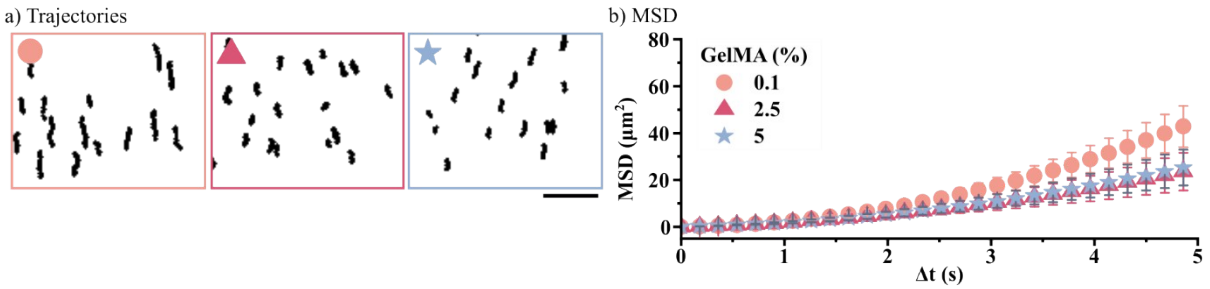

Figure S7. Trajectory maps (a) and MSD plots (b) of $^{1}M^{Coll}$ in hydrogels made from 0.1, 2.5, or 5 % GelMA using 2 mM calcium as the trigger. Scale bar is 100 µm.

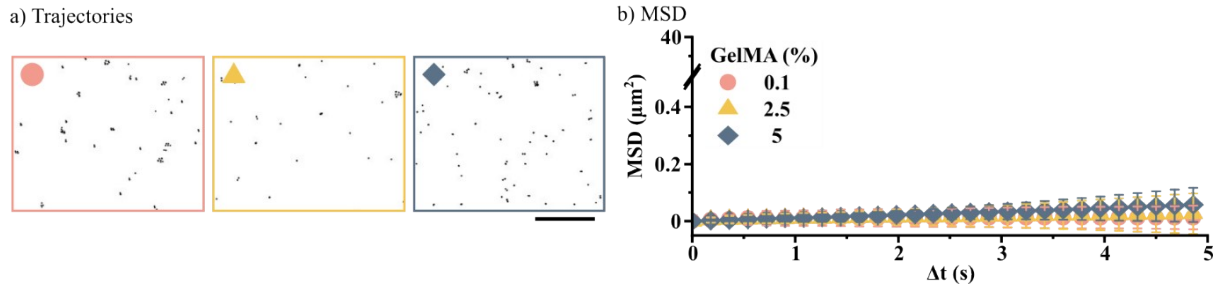

Figure S8. Trajectory maps (a) and MSD plots (b) of $^{2}M^{Coll}$ in hydrogels made from 0.1, 2.5, or 5 % GelMA using 2 mM calcium as the trigger. Scale bar is 50 µm.

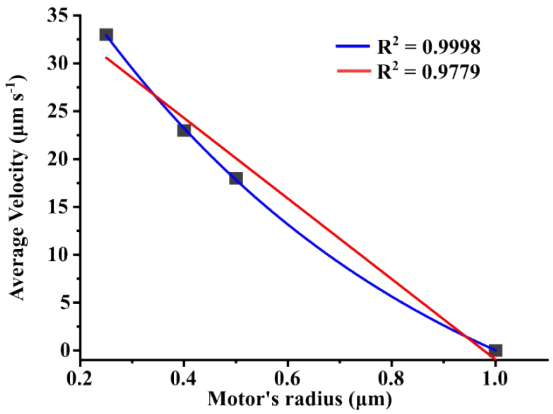

Figure S9. Plot of velocity vs. motor's radius comparing a linear (red) and an exponential fit (blue). Theory predicts a linear dependence of velocity and particle radius for slow particles moving in continuous fluids, while a non-linear dependence is expected for fast particles and complex environments.

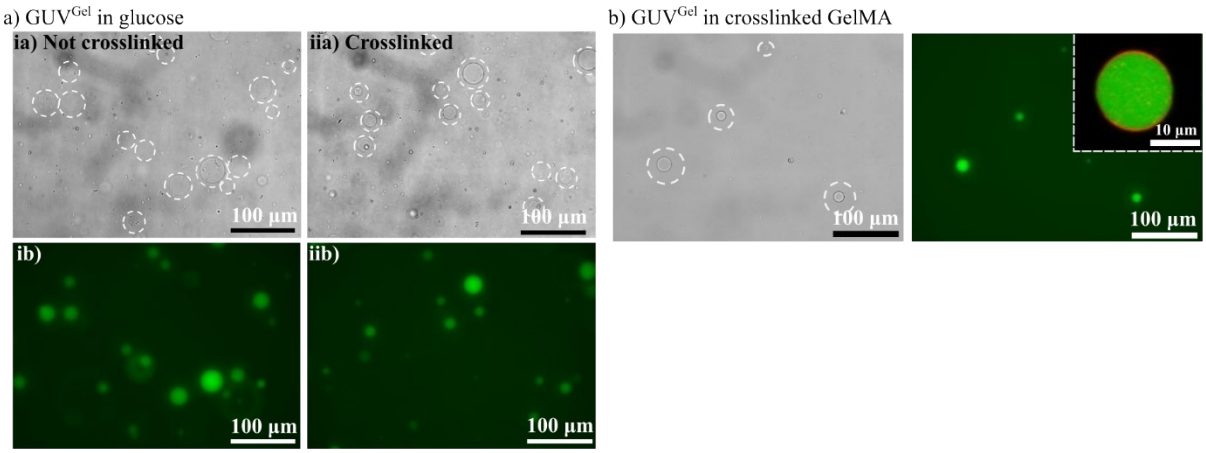

Figure S10. a) Representative fluorescence inverted microscope image of GUV^{Gel} in glucose buffer before and after UV crosslinking. b) Representative fluorescence inverted microscope image of GUV^{Gel} in crosslinked gelatine.