

Supplementary Material

# Preparation and In Vitro Characterization of *Lactococcus lactis*-Loaded Alginate Particles as a Promising Delivery Tool for Periodontal Probiotic Therapy

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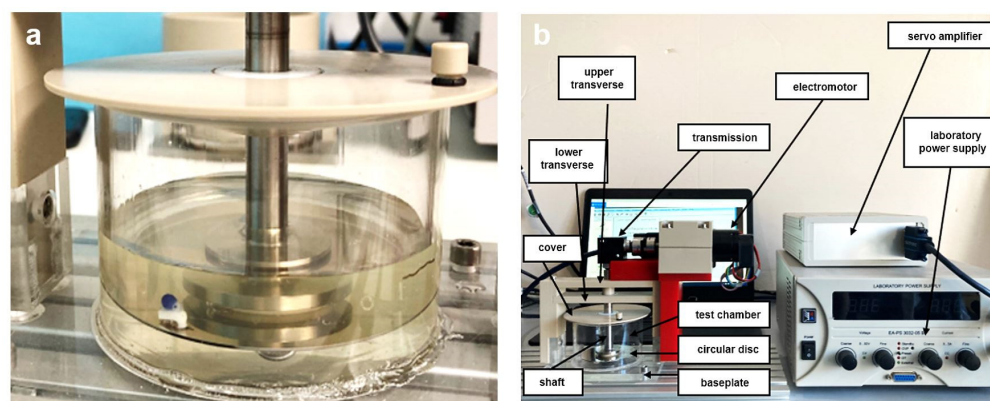
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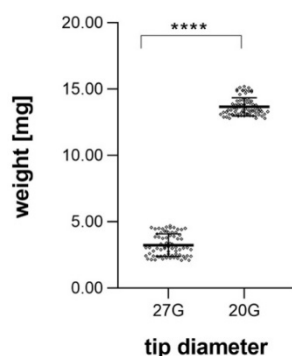
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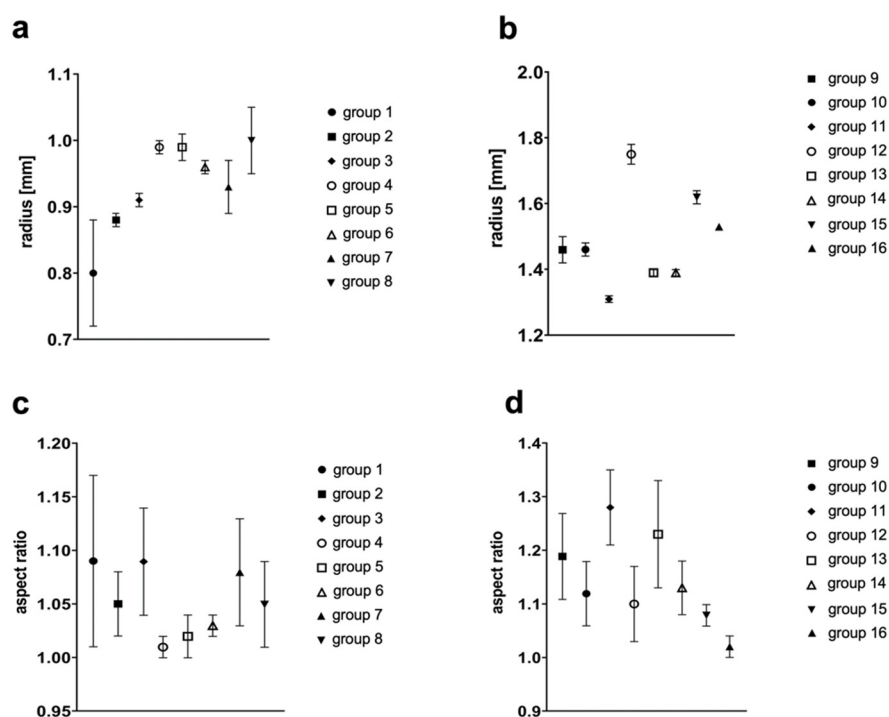
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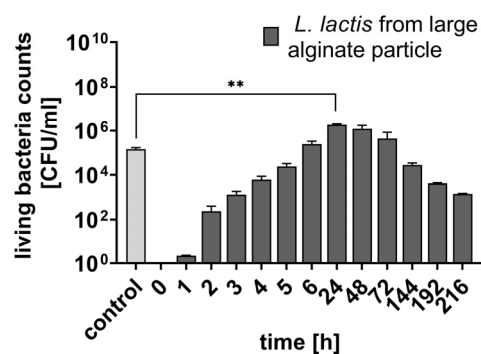
**Figure S1.** Construction of the spinning disk device. (a) Image demonstrates the laminar flow box filled with artificial saliva. The alginate particle is stained blue and attached to a tooth specimen on a circular disk. (b) Technical components for the *in vitro* measurement of the shear force.



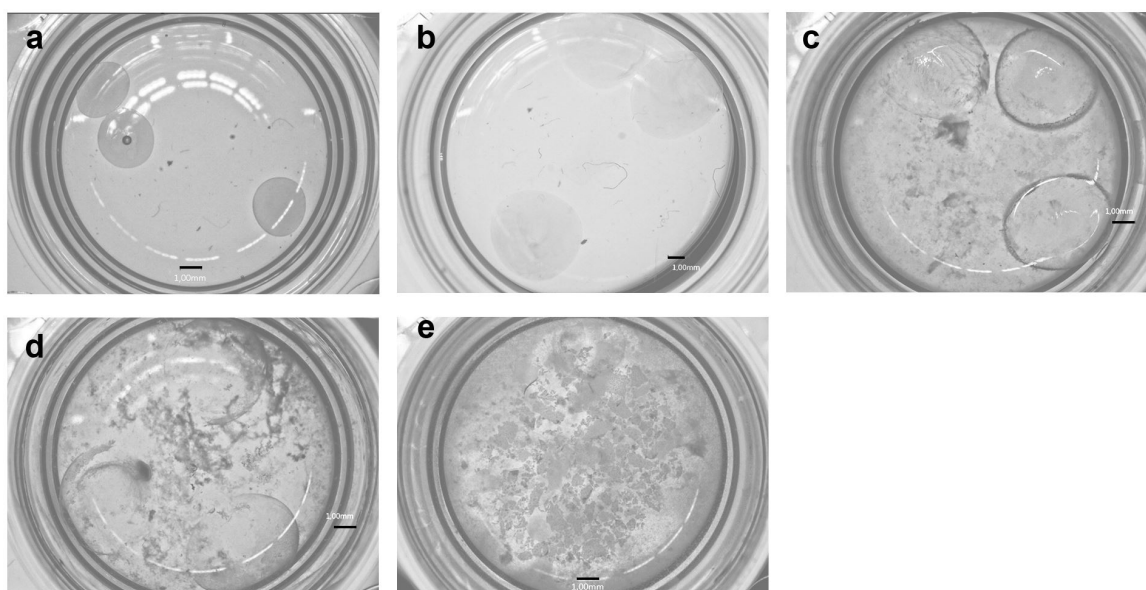
**Figure S2.** Effect of the different dripping tip size (27G vs. 20G) on the weight of alginate particles. Data represent mean  $\pm$  SD of  $n = 72$  analyzed particles per condition from 24 independent experiments with 3 technical replicates per experiment (\*\*\*\* $p < 0.0001$  analyzed with unpaired t-test).



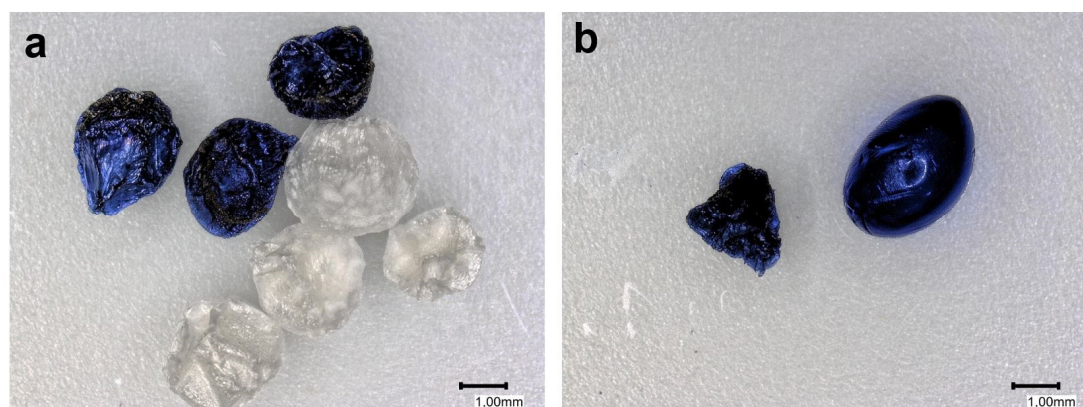
**Figure S3.** Sample distribution across analyzed groups in terms of size (radius) and roundness (aspect ratio) of alginate particles. (a) The technical pre-settings applied to groups No. 1 to 8 produced particles under 1.1 mm in radius, whereas settings for (b) groups No. 9 to 16 produced particles larger than 1.2 mm. (c,d) Representation of the effect of the different pre-settings on the aspect ratio in each group. Data shown as mean  $\pm$  SD of  $n = 9$  particles per condition from 3 independent experiments with 3 technical replicates per experiment.



**Figure S4.** Comparison of released amount of *L. lactis* from a large particle to initially encapsulated quantity. Data represent mean  $\pm$  SD of  $n = 15$  analyzed particles per condition from 3 independent experiments with 5 technical replicates per experiment (\*\*  $p < 0.01$  analyzed with unpaired t-test).

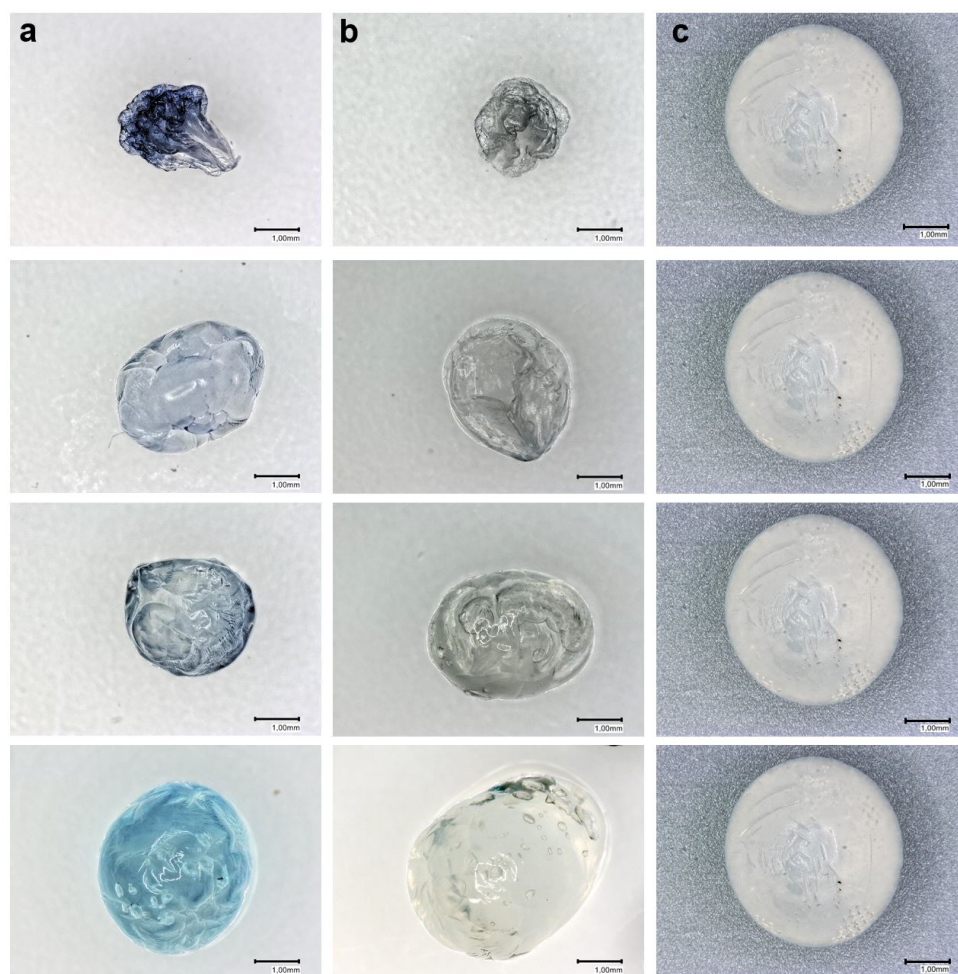


**Figure S5. Digital microscopic analysis of the stability of alginate particle in artificial saliva.** (a) Microscopic image of  $n = 3$  alginate particles (group No. 16) directly after insertion into artificial saliva. (b) Particles appeared considerably enlarged after 5 days incubation. (c) Incipient degradation was detected after 10 days incubation. (d) 15 days of incubation led to visible disintegration of particles. (e) Particles were fully dissolved after 20 days incubation in artificial saliva. Magnification: 20 $\times$ .

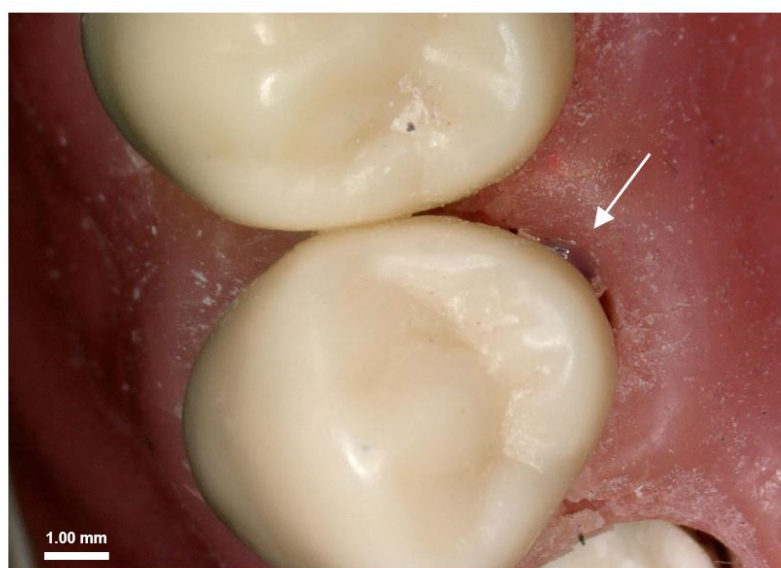


**Figure S6. Digital microscopic analysis of lyophilized vs. non-lyophilized particle.** (a) Lyophilized particles, three of them stained in trypan blue for an optimal visualization. Magnification: 30 $\times$ . (b) Lyophilized particle (left) in comparison to non-lyophilized reference particle (right). Magnification: 30 $\times$ .





**Figure S7. Digital microscopic analysis of the swelling behavior of lyophilized alginate particles.** Lyophilized particles were rehydrated in (a) artificial saliva or (b) water and compared to (c) non-lyophilized reference particles. Images were taken after 1 h, 2 h, 4 h and 24 h of incubation. Scale = 1 mm. Magnification: 50 $\times$ .



**Figure S8. Digital microscopic image exemplifying future clinical application of an alginate particle.** An alginate particle loaded with *L. lactis* is placed in a lingual periodontal pocket (tooth 44) on a phantom model of the human jaw. Magnification: 10 $\times$ .