Electronic Supplementary Material (ESI) for Nanoscale Advances.


# Hydrophobic soot nanoparticles as a non-cytotoxic motility activator of human spermatozoa

Supporting information

Karekin D. Esmeryan[a]*, Ivaylo Rangelov[b] and Todor A. Chaushev[b]

[a]Acoustoelectronics Laboratory, Georgi Nadjakov Institute of Solid State Physics, Bulgarian Academy of Sciences, 72, Tzarigradsko Chaussee Blvd., 1784 Sofia, Bulgaria

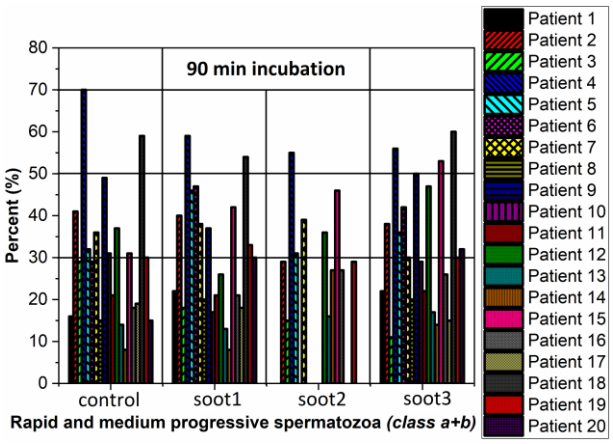
[b]Research Department, Medical Center Neovitro OOD, 20, Petko Y. Todorov Blvd., 1408 Sofia, Bulgaria

*Corresponding author: tel. +359 2 979 5811; e-mail: karekin_esmerian@abv.bg

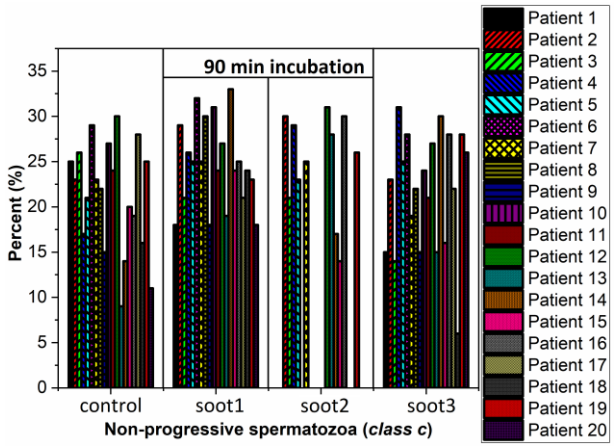
**Table S1:** Sperm motility after prolonged incubation in vials without (control sample – C) and with different types of soot (S1 – *soot1*; S2 – *soot2*; S3 – *soot3*). The blue and red colors indicate negative and positive effects of the soot towards the sperm motility, respectively. The normal font denotes lack of substantial differences between the control and soot-seminal suspensions.

| ***Patient*** | ***Sperm status*** | ***Motility class (%)*** | ***fresh*** | ***90 min*** | | | | ***180 min*** | | | | ***270 min*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ***C*** | ***S1*** | ***S2*** | ***S3*** | ***C*** | ***S1*** | ***S2*** | ***S3*** | ***C*** | ***S1*** | ***S2*** | ***S3*** |
| 1 | Astheno | ***a+b*** | 27 | 16 | **22** | - | **22** | 17 | **13** | - | **12** | 12 | **17** | - | **15** |
| | | ***c*** | 17 | 25 | **18** | - | **15** | 20 | **27** | - | **24** | 20 | **17** | - | **16** |
| | | ***d*** | 56 | 59 | 60 | - | **63** | 63 | **60** | - | 64 | 68 | 66 | - | 69 |
| 2 | Normo | ***a+b*** | 60 | 41 | 40 | **29** | **38** | 38 | 40 | **34** | **41** | 27 | **31** | 26 | 29 |
| | | ***c*** | 20 | 23 | **29** | **30** | 23 | 33 | **17** | **30** | **16** | 36 | 38 | **29** | **13** |
| | | ***d*** | 20 | 36 | **31** | **41** | **39** | 29 | **43** | **36** | **43** | 37 | **31** | **45** | **58** |
| 3 | Normo | ***a+b*** | 58 | 29 | **18** | **15** | **11** | 14 | **7** | **10** | **7** | - | - | - | - |
| | | ***c*** | 22 | 26 | **21** | **21** | **14** | 17 | 16 | 15 | **11** | - | - | - | - |
| | | ***d*** | 20 | 45 | **61** | **64** | **75** | 69 | **77** | **75** | **82** | - | - | - | - |
| 4 | Normo | ***a+b*** | 59 | 70 | **59** | **55** | **56** | 37 | **18** | 37 | **31** | 14 | **11** | **21** | 16 |
| | | ***c*** | 25 | 17 | **26** | **29** | **31** | 36 | **31** | 36 | **33** | 32 | **26** | **36** | **35** |
| | | ***d*** | 16 | 13 | 15 | **16** | 13 | 27 | **51** | 27 | **36** | 54 | **63** | **43** | **49** |
| 5 | Normo | ***a+b*** | 68 | 32 | **46** | 31 | **36** | 25 | **29** | **20** | **15** | 10 | **18** | 10 | 9 |
| | | ***c*** | 17 | 21 | **25** | 23 | **25** | 24 | 26 | 22 | **31** | 14 | **26** | **22** | **26** |
| | | ***d*** | 15 | 47 | **29** | 46 | **39** | 51 | **45** | **58** | **54** | 76 | **56** | **68** | **65** |
| 6 | Normo | ***a+b*** | 52 | 29 | **47** | - | **42** | 27 | **32** | - | **43** | 15 | **41** | - | **23** |
| | | ***c*** | 26 | 29 | **32** | - | 28 | 39 | **34** | - | **30** | 29 | 31 | - | **26** |
| | | ***d*** | 22 | 42 | **21** | - | **30** | 34 | 34 | - | **27** | 56 | **28** | - | **51** |
| 7 | Normo | ***a+b*** | 60 | 36 | 38 | **39** | **30** | 12 | **5** | 14 | **18** | 5 | 4 | **9** | **9** |
| | | ***c*** | 21 | 23 | 25 | 25 | **19** | 22 | **15** | 24 | **25** | 28 | **24** | **25** | **22** |
| | | ***d*** | 19 | 41 | **37** | **36** | **51** | 66 | **80** | **62** | **57** | 67 | **72** | 66 | 69 |
| 8 | Normo | ***a+b*** | 39 | 15 | **20** | - | **20** | 11 | 13 | - | **8** | 11 | **14** | - | **38** |
| | | ***c*** | 27 | 22 | **30** | - | 22 | 24 | 26 | - | **14** | 26 | **33** | - | **16** |
| | | ***d*** | 34 | 63 | **50** | - | **58** | 65 | **61** | - | **78** | 63 | **53** | - | **46** |
| 9 | Normo | ***a+b*** | 50 | 49 | **37** | - | 50 | 36 | **33** | - | **28** | 14 | 12 | - | **11** |
| | | ***c*** | 16 | 15 | **18** | - | 15 | 23 | 22 | - | 21 | 23 | 22 | - | 22 |
| | | ***d*** | 34 | 36 | **45** | - | 35 | 41 | **45** | - | **51** | 63 | **66** | - | **67** |
| 10 | Normo | ***a+b*** | 55 | 31 | **17** | - | 29 | 44 | **25** | - | **26** | 29 | **18** | - | **22** |
| | | ***c*** | 26 | 27 | **31** | - | **24** | 12 | **28** | - | **31** | 14 | **21** | - | **38** |
| | | ***d*** | 19 | 42 | **52** | - | **47** | 44 | **47** | - | 43 | 57 | **61** | - | **40** |
| 11 | Normo | ***a+b*** | 41 | 21 | 21 | - | 22 | 17 | **27** | - | **26** | 9 | **23** | - | **29** |
| | | ***c*** | 30 | 24 | 24 | - | 21 | 29 | **21** | - | **17** | 24 | **29** | - | 23 |
| | | ***d*** | 29 | 55 | 55 | - | 57 | 54 | 52 | - | **57** | 67 | **48** | - | **48** |
| 12 | Normo | ***a+b*** | 63 | 37 | **26** | 36 | **47** | 14 | **28** | **18** | **22** | - | - | - | - |
| | | ***c*** | 19 | 30 | **27** | 31 | **27** | 40 | **37** | **29** | **30** | - | - | - | - |
| | | ***d*** | 18 | 33 | **47** | 33 | **26** | 46 | **35** | **53** | 48 | - | - | - | - |

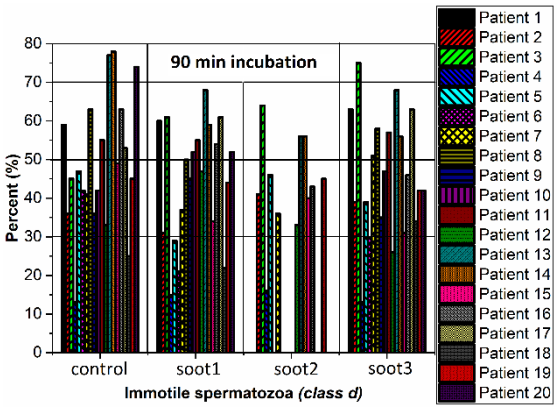
| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | Oligo-astheno | ***a+b*** | 28 | 14 | 13 | 16 | **17** | 14 | **6** | **11** | 15 | 5 | **0** | **0** | **12** |
| | | ***c*** | 7 | 9 | **19** | **28** | **15** | 9 | **26** | **19** | **20** | 12 | **3** | **5** | **18** |
| | | ***d*** | 65 | 77 | **68** | **56** | **68** | 77 | **68** | **70** | **65** | 83 | **97** | **95** | **70** |
| 14 | Normo | ***a+b*** | 59 | 8 | 8 | **27** | **14** | 4 | 5 | **17** | **7** | - | - | - | - |
| | | ***c*** | 25 | 14 | **33** | **17** | **30** | 18 | **26** | **25** | **24** | - | - | - | - |
| | | ***d*** | 16 | 78 | **59** | **56** | **56** | 78 | **69** | **58** | **69** | - | - | - | - |
| 15 | Normo | ***a+b*** | 51 | 31 | **42** | **46** | **53** | 6 | 7 | **17** | **21** | 3 | 3 | **6** | **8** |
| | | ***c*** | 21 | 20 | **24** | **14** | **16** | 25 | **28** | **19** | 24 | 19 | **13** | 18 | 17 |
| | | ***d*** | 28 | 49 | **34** | **40** | **31** | 69 | **65** | **64** | **55** | 78 | **84** | 76 | **75** |
| 16 | Astheno | ***a+b*** | 31 | 18 | **21** | **27** | **26** | 20 | **16** | **16** | **14** | - | - | - | - |
| | | ***c*** | 27 | 19 | **25** | **30** | **28** | 24 | **29** | 24 | **21** | - | - | - | - |
| | | ***d*** | 42 | 63 | **54** | **43** | **46** | 56 | 55 | **60** | **65** | - | - | - | - |
| 17 | Oligo | ***a+b*** | 46 | 19 | 18 | - | **15** | 5 | **16** | - | 3 | 2 | 2 | - | 2 |
| | | ***c*** | 9 | 28 | **21** | - | **22** | 27 | **21** | - | **17** | 14 | 16 | - | **11** |
| | | ***d*** | 45 | 53 | **61** | - | **63** | 68 | **63** | - | **80** | 84 | 82 | - | **87** |
| 18 | Oligo | ***a+b*** | 48 | 59 | **54** | - | 60 | 22 | **35** | - | **27** | 7 | **20** | - | **23** |
| | | ***c*** | 15 | 16 | **24** | - | **6** | 36 | **12** | - | **19** | 32 | **18** | - | **26** |
| | | ***d*** | 37 | 25 | **22** | - | **34** | 42 | **53** | - | **54** | 61 | 62 | - | **51** |
| 19 | Astheno | ***a+b*** | 21 | 30 | **33** | 29 | 30 | 15 | **20** | **18** | 16 | - | - | - | - |
| | | ***c*** | 27 | 25 | 23 | 26 | **28** | 28 | **36** | 29 | 28 | - | - | - | - |
| | | ***d*** | 52 | 45 | 44 | 45 | **42** | 57 | **44** | **53** | 56 | - | - | - | - |
| 20 | Astheno | ***a+b*** | 24 | 15 | **30** | - | **32** | 24 | **19** | - | **21** | 14 | **17** | - | **26** |
| | | ***c*** | 22 | 11 | **18** | - | **26** | 35 | **30** | - | **21** | 16 | **28** | - | **26** |
| | | ***d*** | 54 | 74 | **52** | - | **42** | 41 | **51** | - | **58** | 70 | **55** | - | **48** |




a)

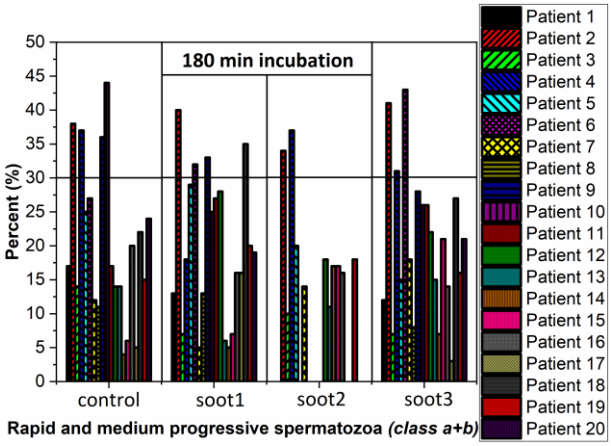



b)

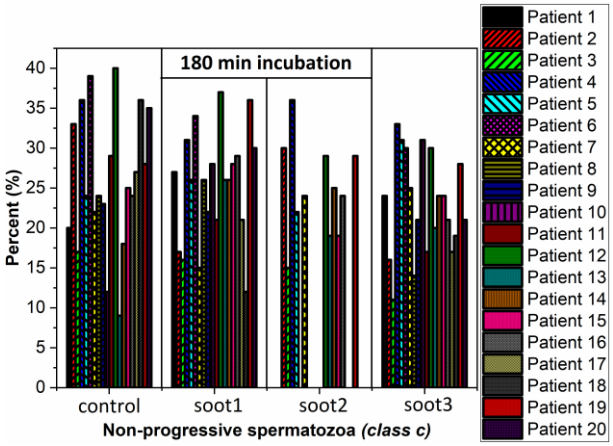



c)

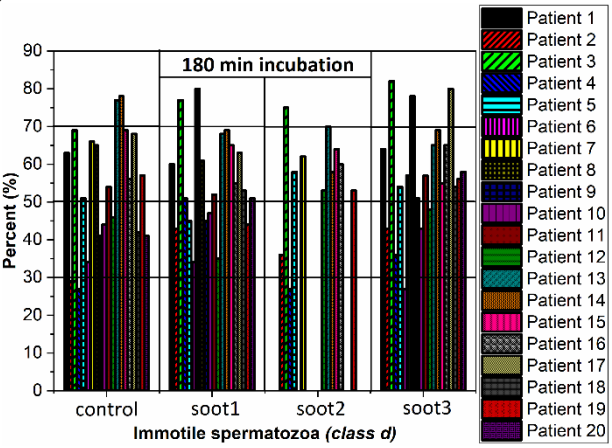
**Figure S1:** Comparison of the percent of a) progressively motile, b) non-progressively motile and c) immotile spermatozoa at twenty patients after 90 min residence in vials without (control) and with *soot1*, *soot2* and *soot3*. The column charts symbolize an integer of the progressively motile, non-progressively motile and immotile species, detected in each particular human ejaculate by the Sperm Class Analyzer software, whose measurement uncertainty is ~1 %.




a)




b)




c)

**Figure S2:** Comparison of the percent of a) progressively motile, b) non-progressively motile and c) immotile spermatozoa at twenty patients after 180 min residence in vials without (control) and

with *soot1*, *soot2* and *soot3*. The column charts symbolize an integer of the progressively motile, non-progressively motile and immotile species, detected in each particular human ejaculate by the Sperm Class Analyzer software, whose measurement uncertainty is ~1 %.

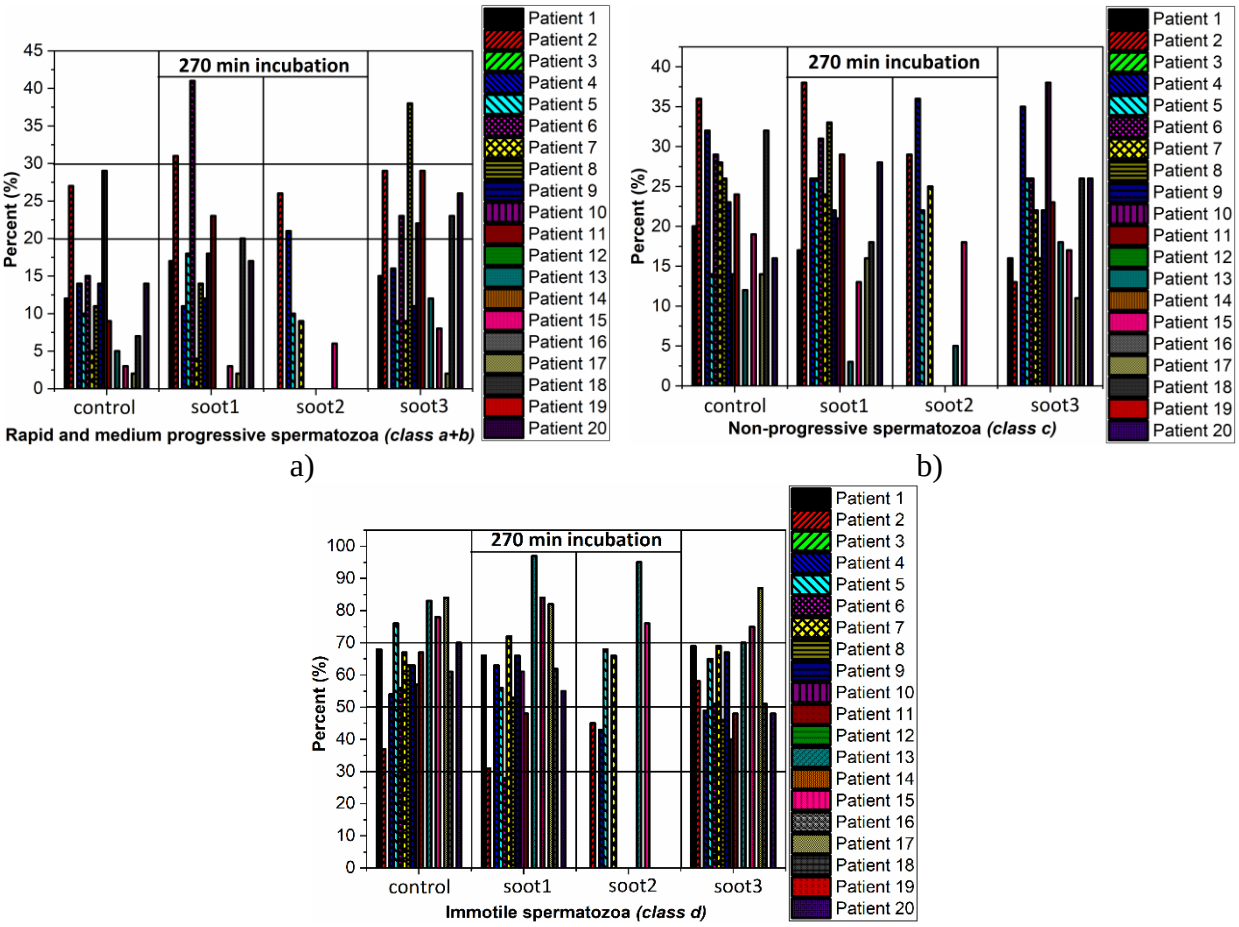



**Figure S3:** Comparison of the percent of a) progressively motile, b) non-progressively motile and c) immotile spermatozoa at fifteen patients after 270 min residence in vials without (control) and with *soot1*, *soot2* and *soot3*. The patients' number is reduced, because the other five individuals not evaluated at that stage possess severely deteriorated seminal parameters of the control sample. The column charts symbolize an integer of the progressively motile, non-progressively motile and immotile species, detected in each particular human ejaculate by the Sperm Class Analyzer software, whose measurement uncertainty is ~1 %.

**Captions to the supporting videos:**

**Video S1 –** sperm motility at patient 14 after 180 min incubation – control sample.

**Video S2 –** sperm motility at patient 14 after 180 min incubation – *soot2*.

**Video S3 –** sperm motility around a soot agglomerate in the beginning of incubation.

**Video S4** – sperm motility around a soot agglomerate after 90 min incubation.