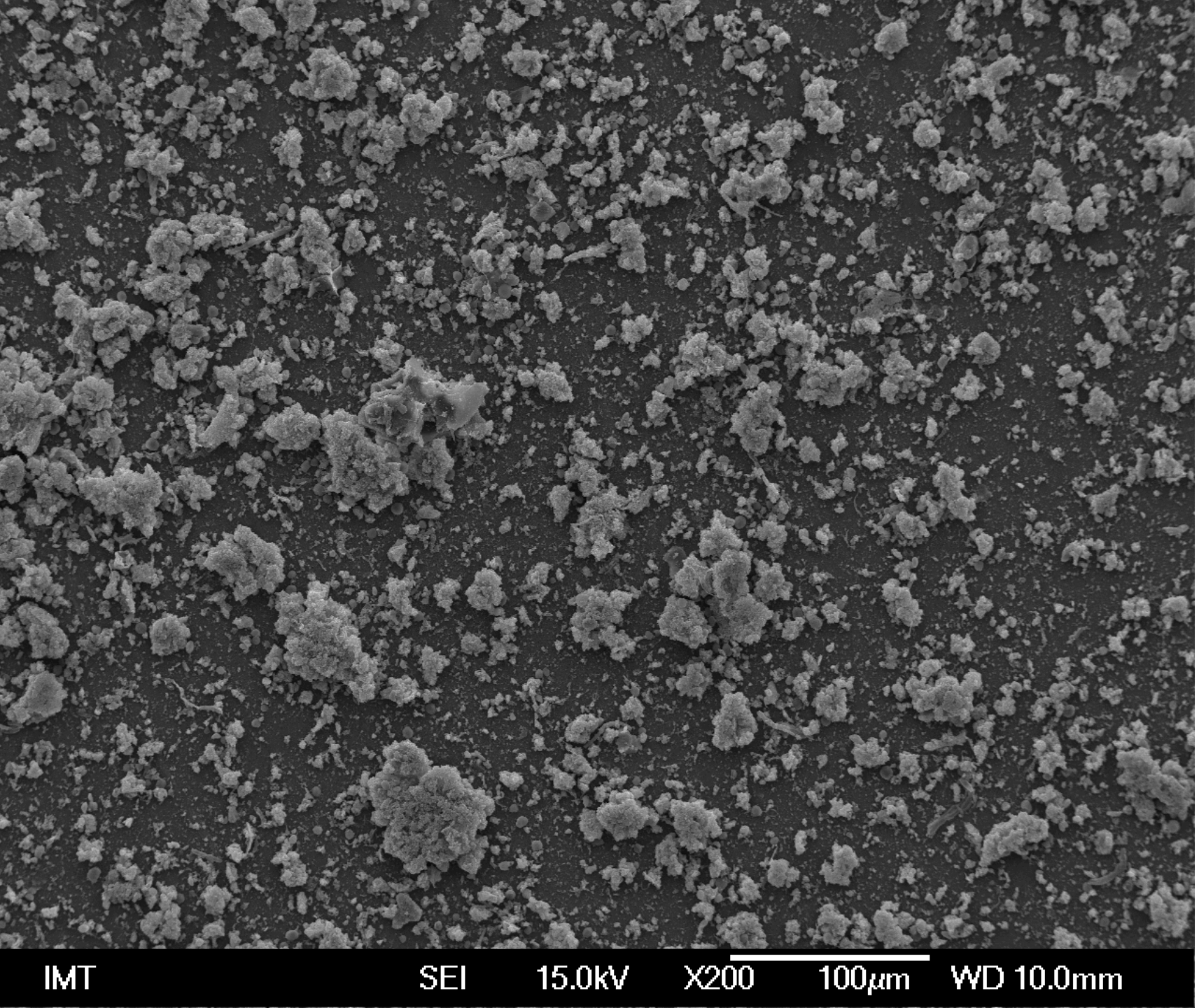
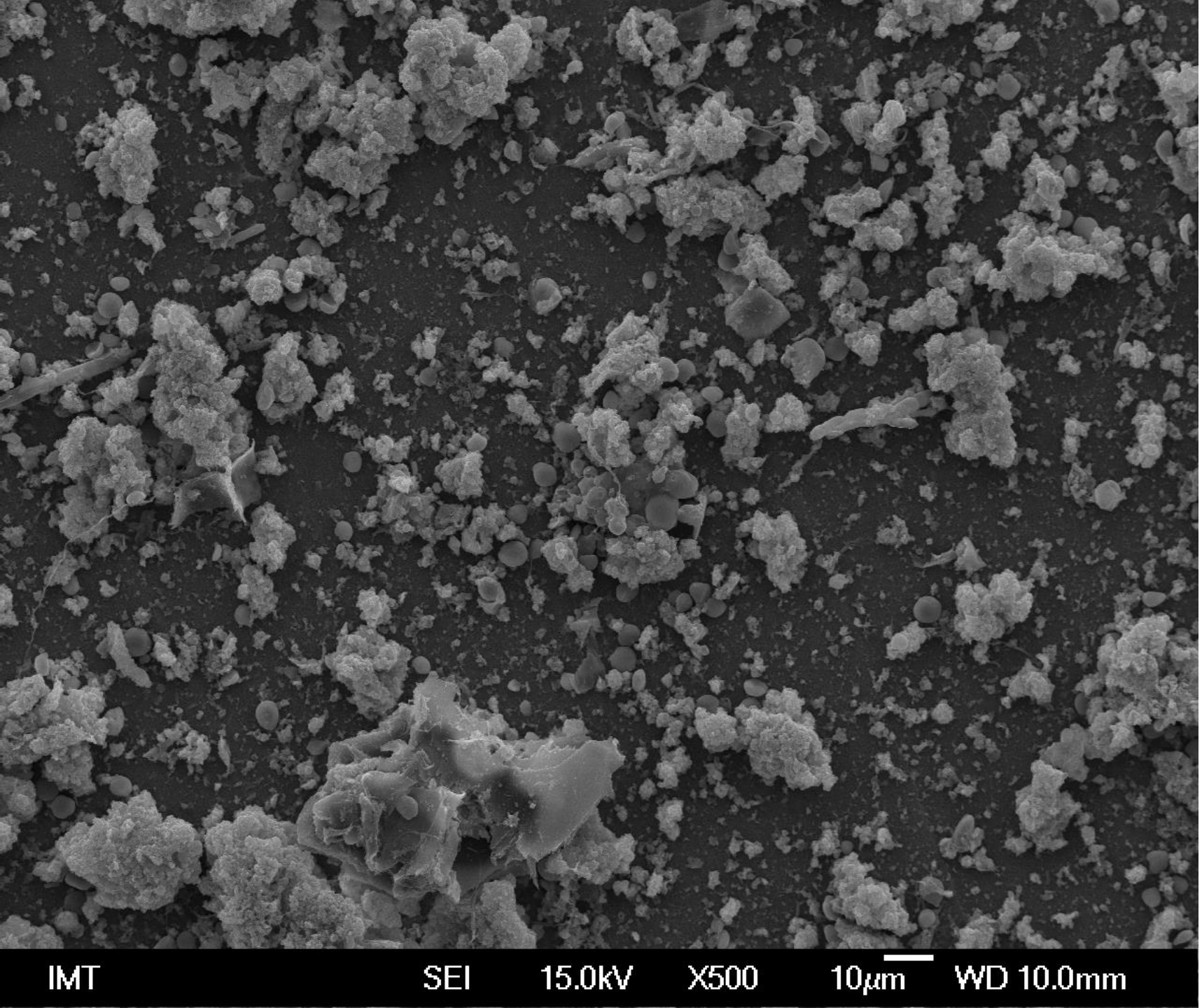


Spruce needle homogenate 1

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

The samples were fixed with OsO4 as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. Int J Mol Sci. 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO4 for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).



Spruce needle homogenate 2

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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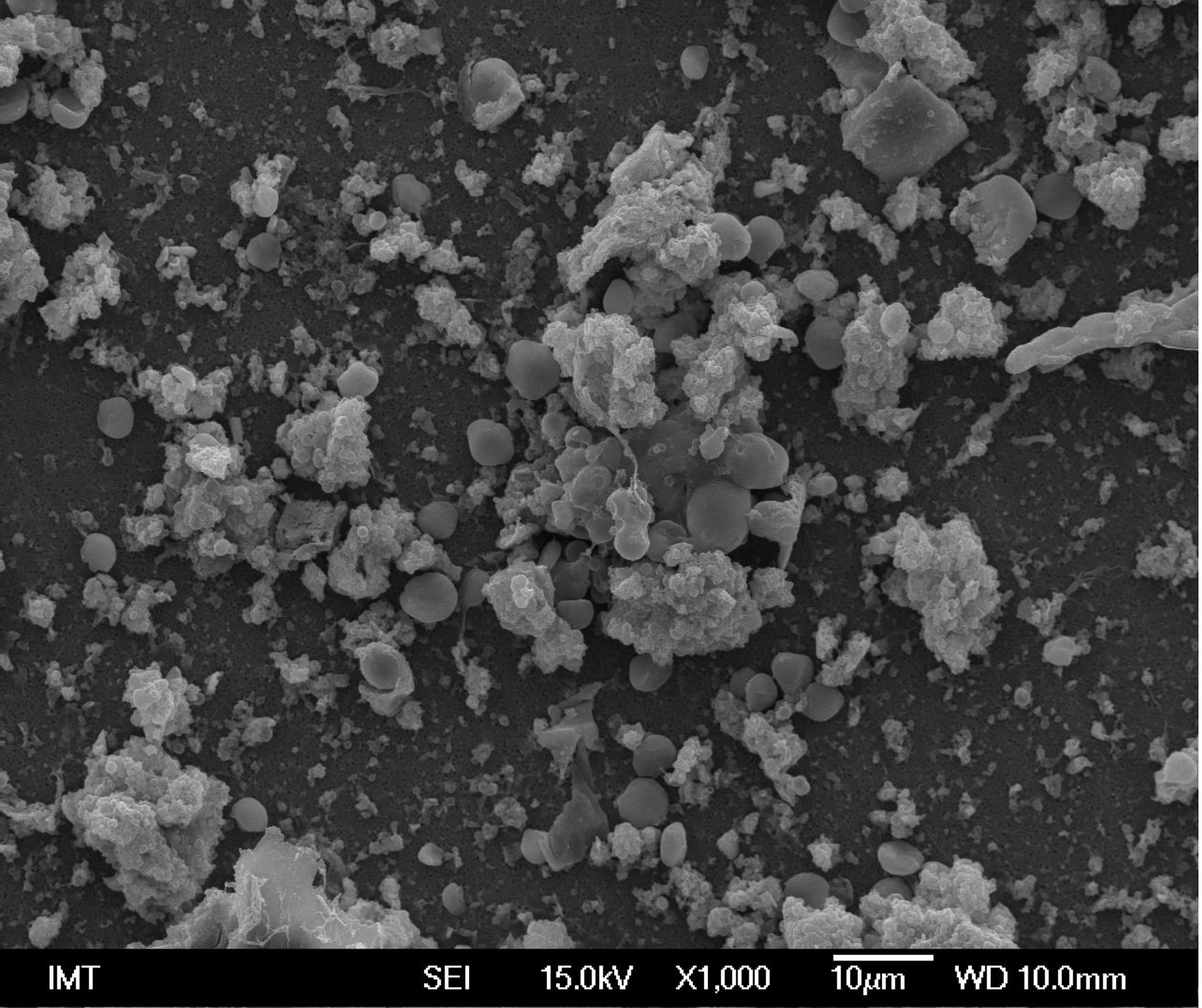
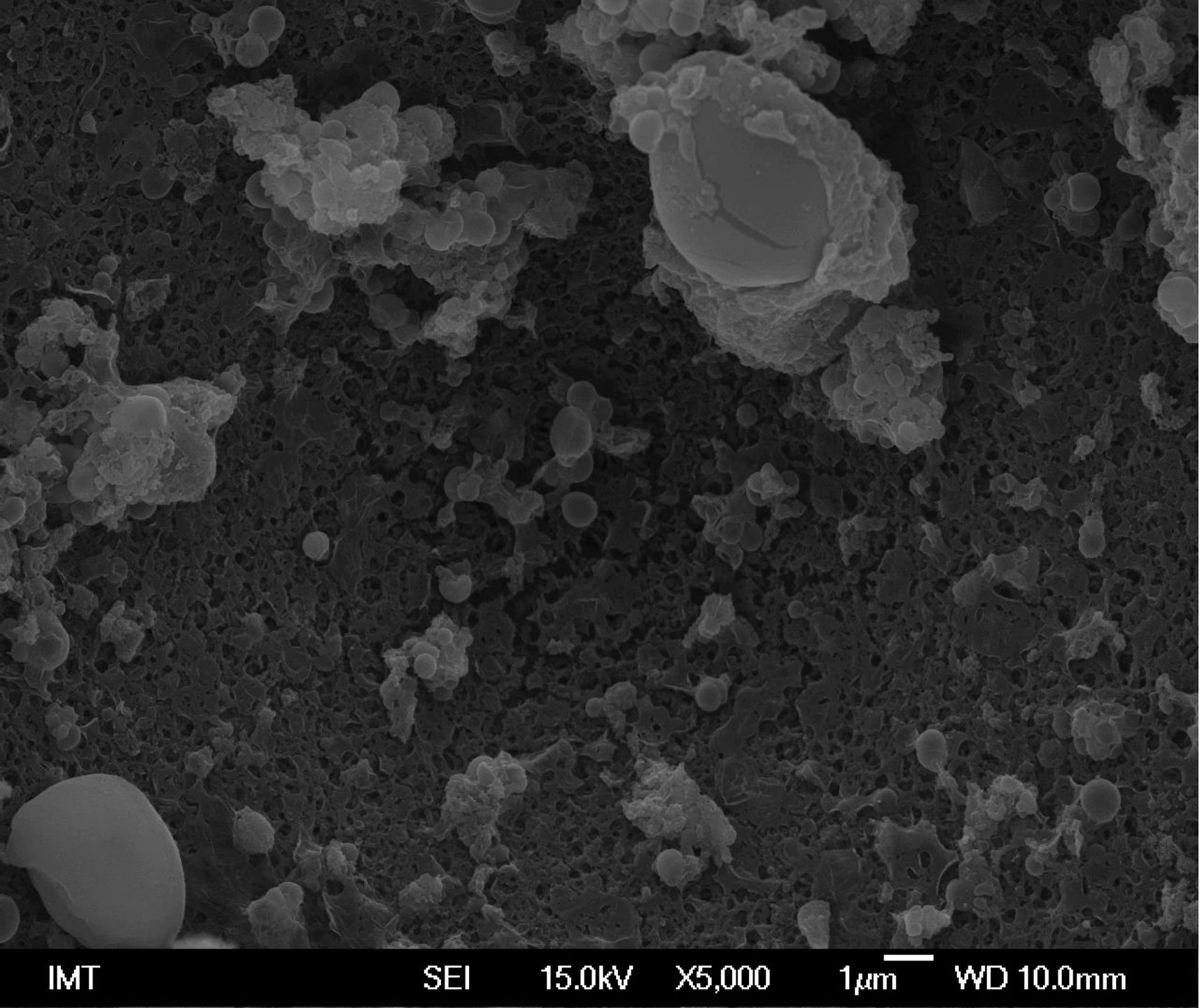


Spruce needle homogenate 3

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

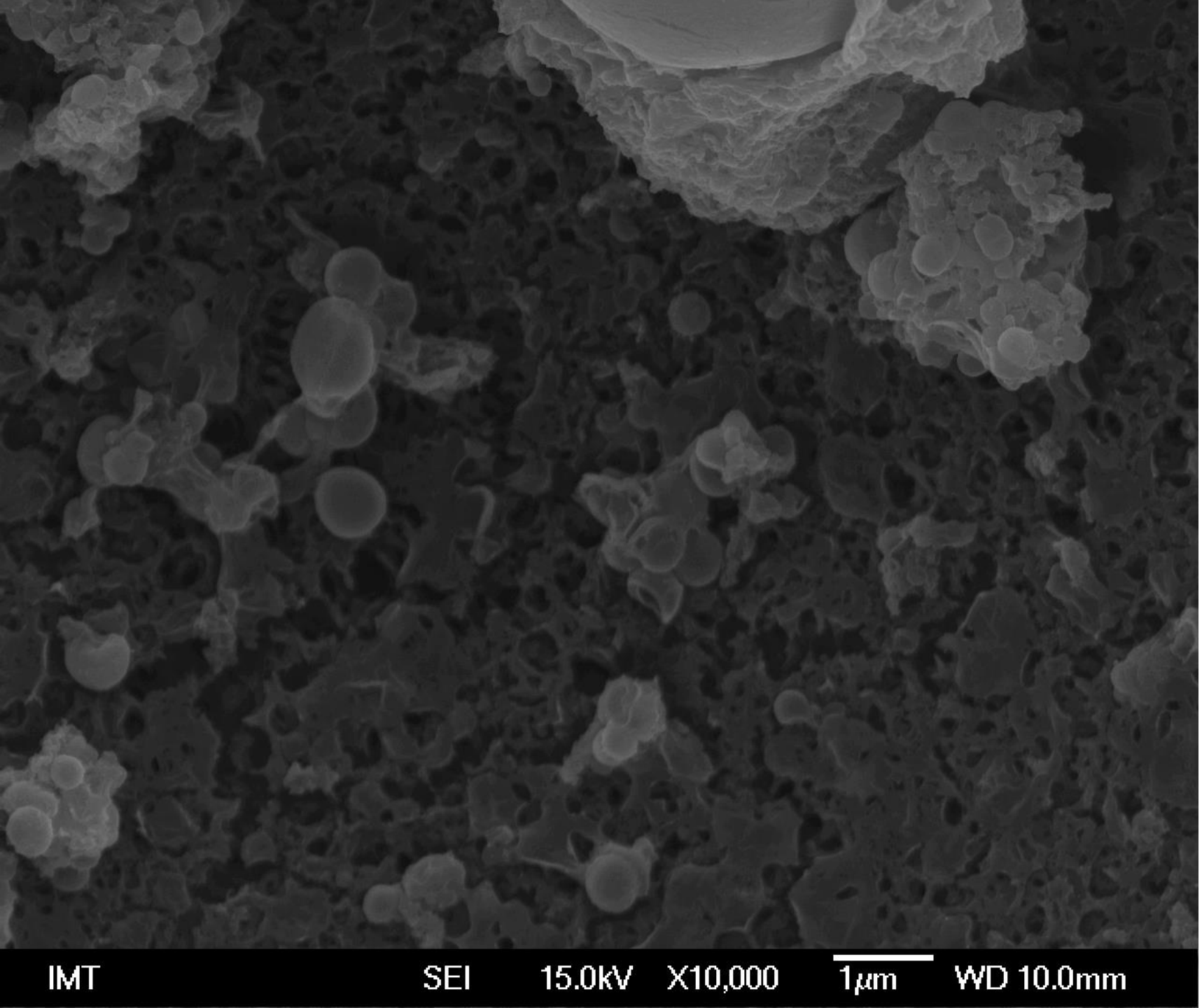
The samples were fixed with OsO_4 as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. Int J Mol Sci. 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO_4 for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).



Spruce needle homogenate 4

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

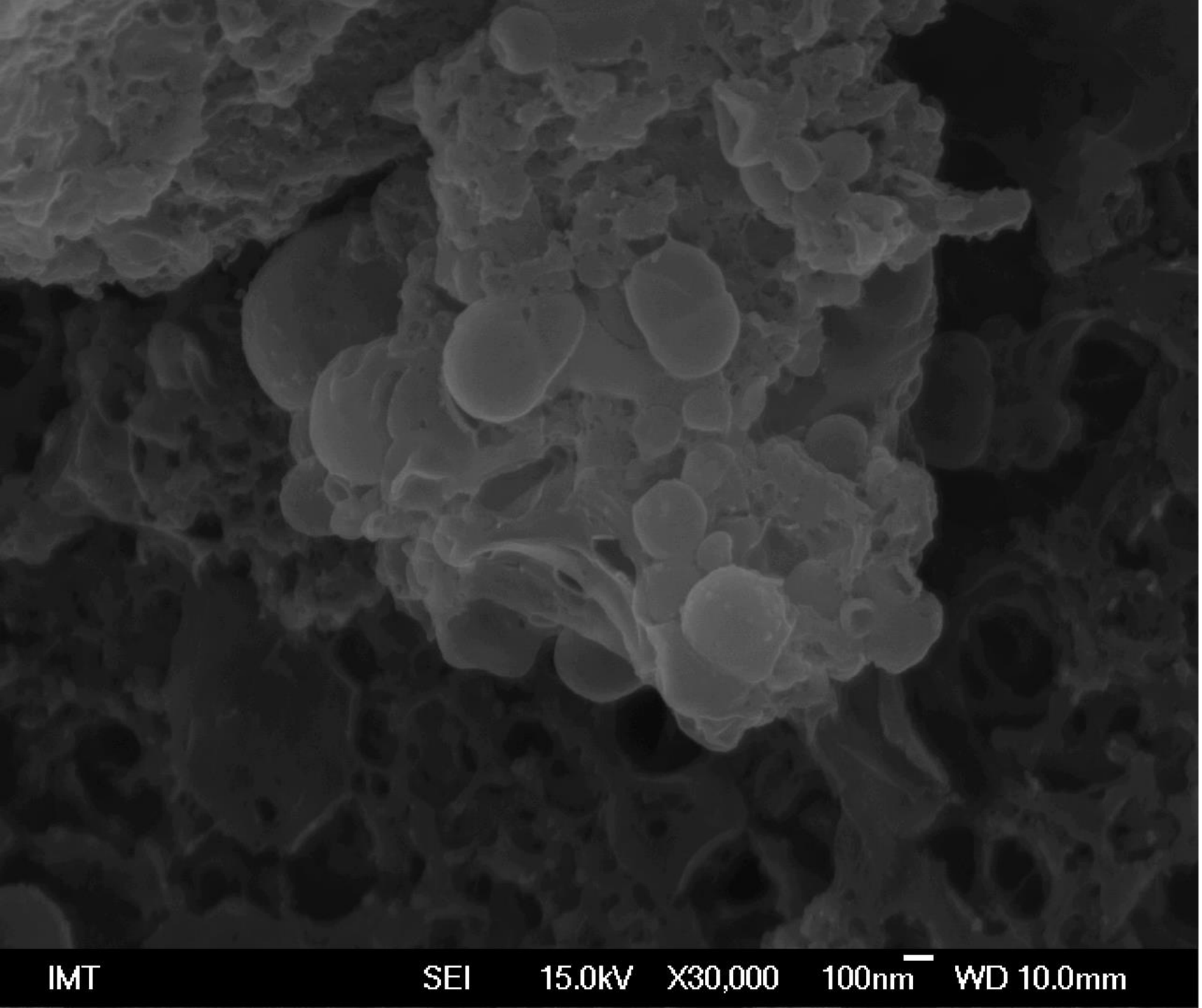
The samples were fixed with OsO_4 as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. Int J Mol Sci. 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO_4 for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).



Spruce needle homogenate 5

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

The samples were fixed with OsO4 as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. Int J Mol Sci. 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO4 for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).



Spruce needle homogenate 6

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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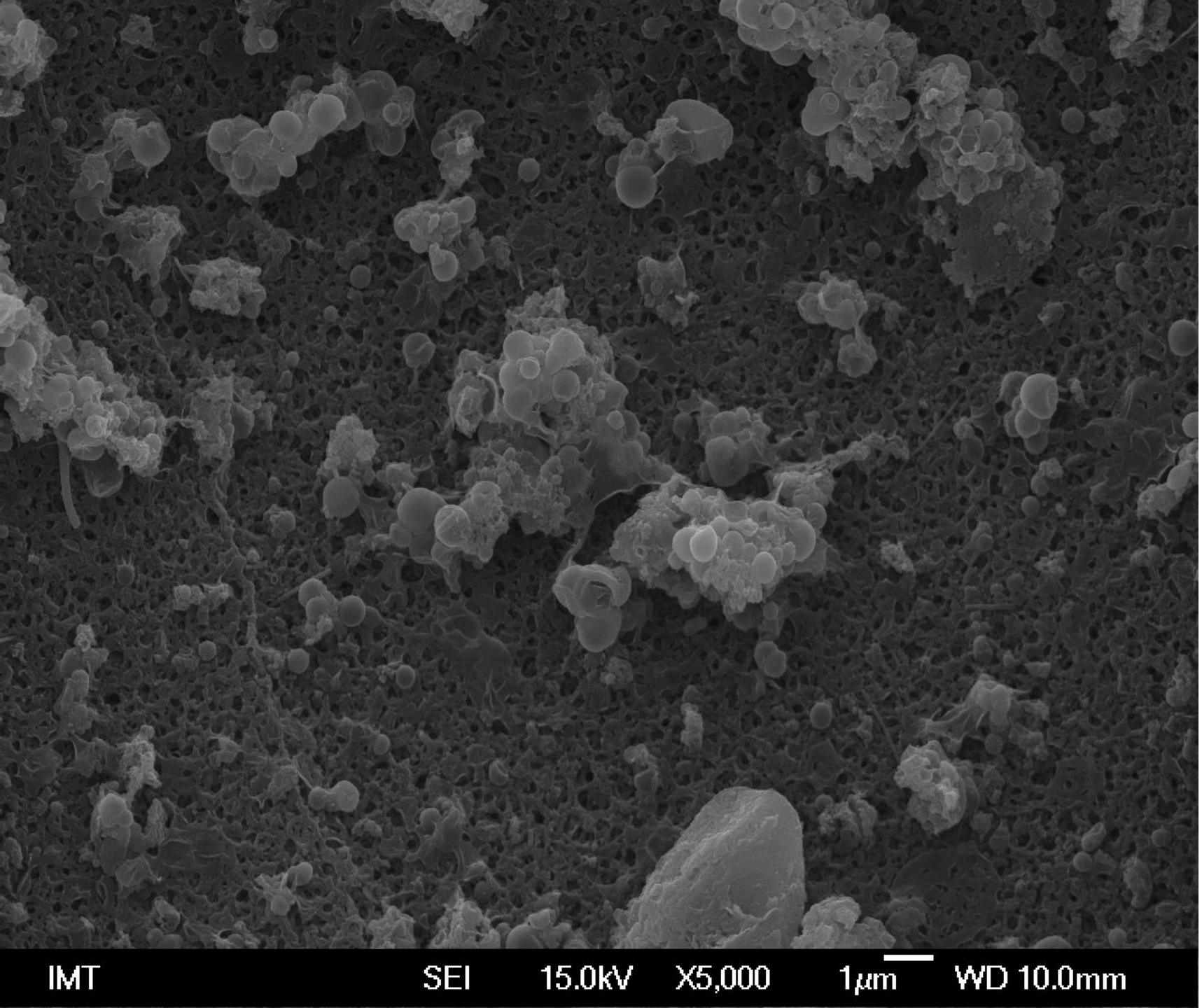

Spruce needle homogenate 7

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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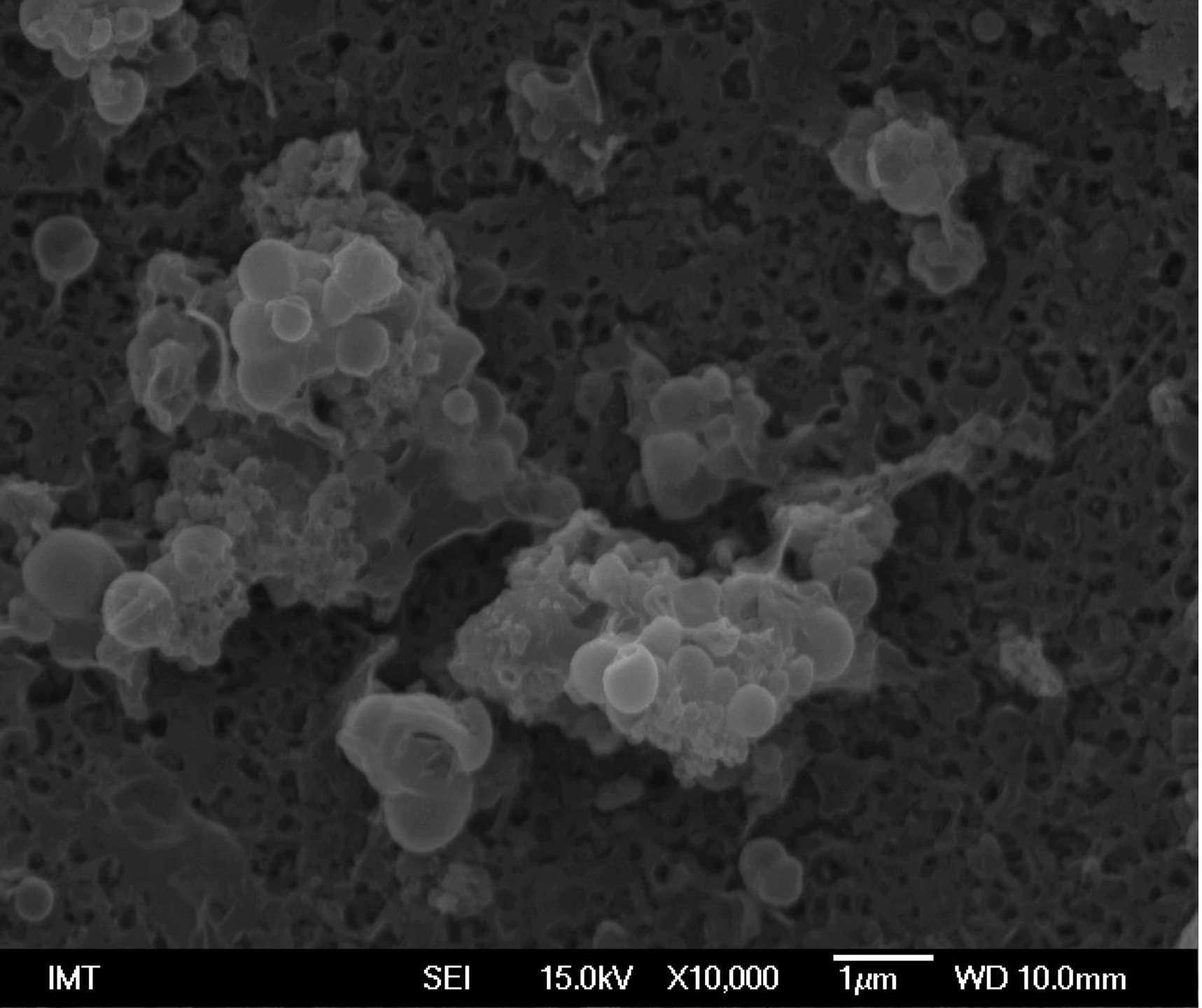

Spruce needle homogenate 8

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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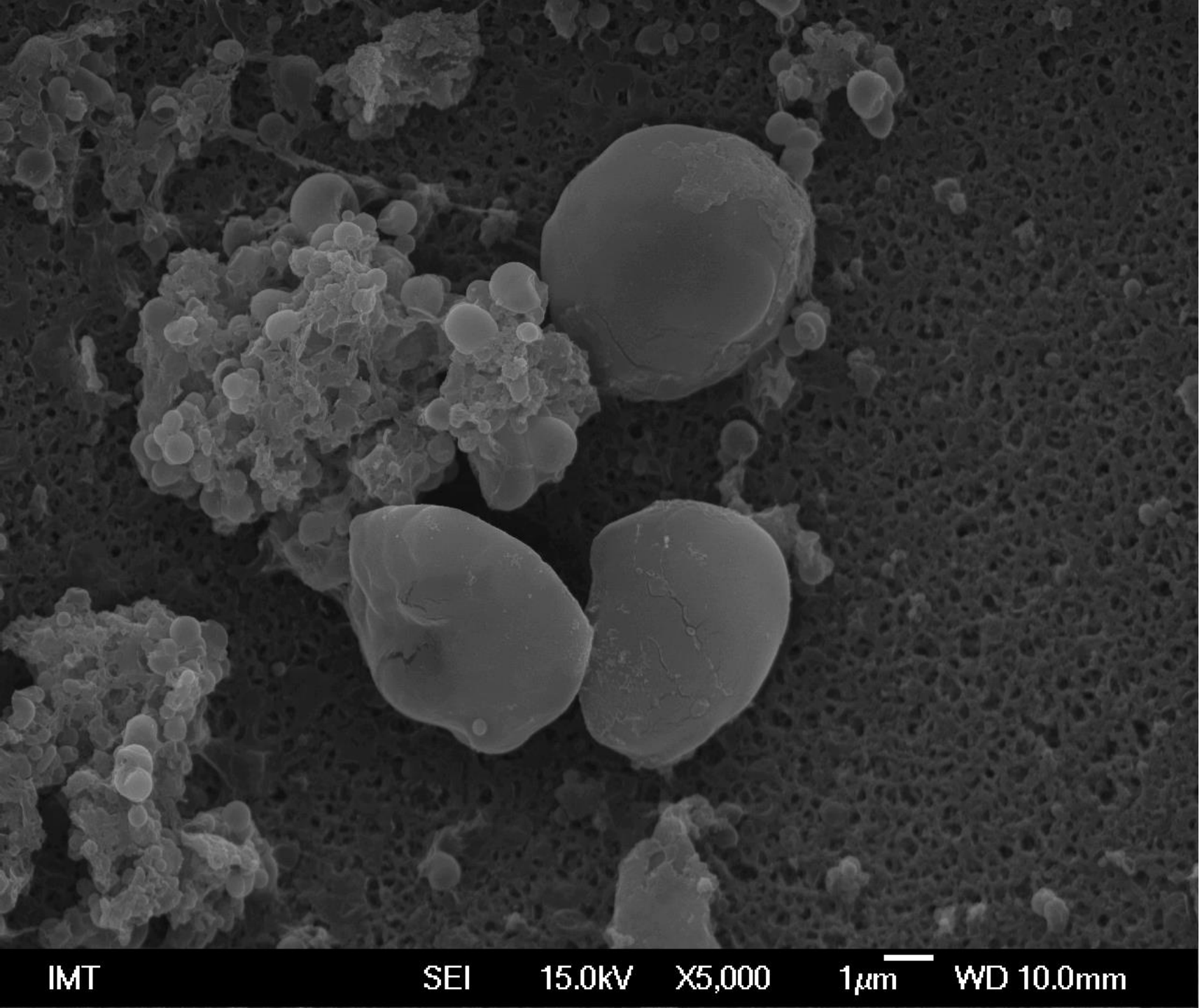

Spruce needle homogenate 9

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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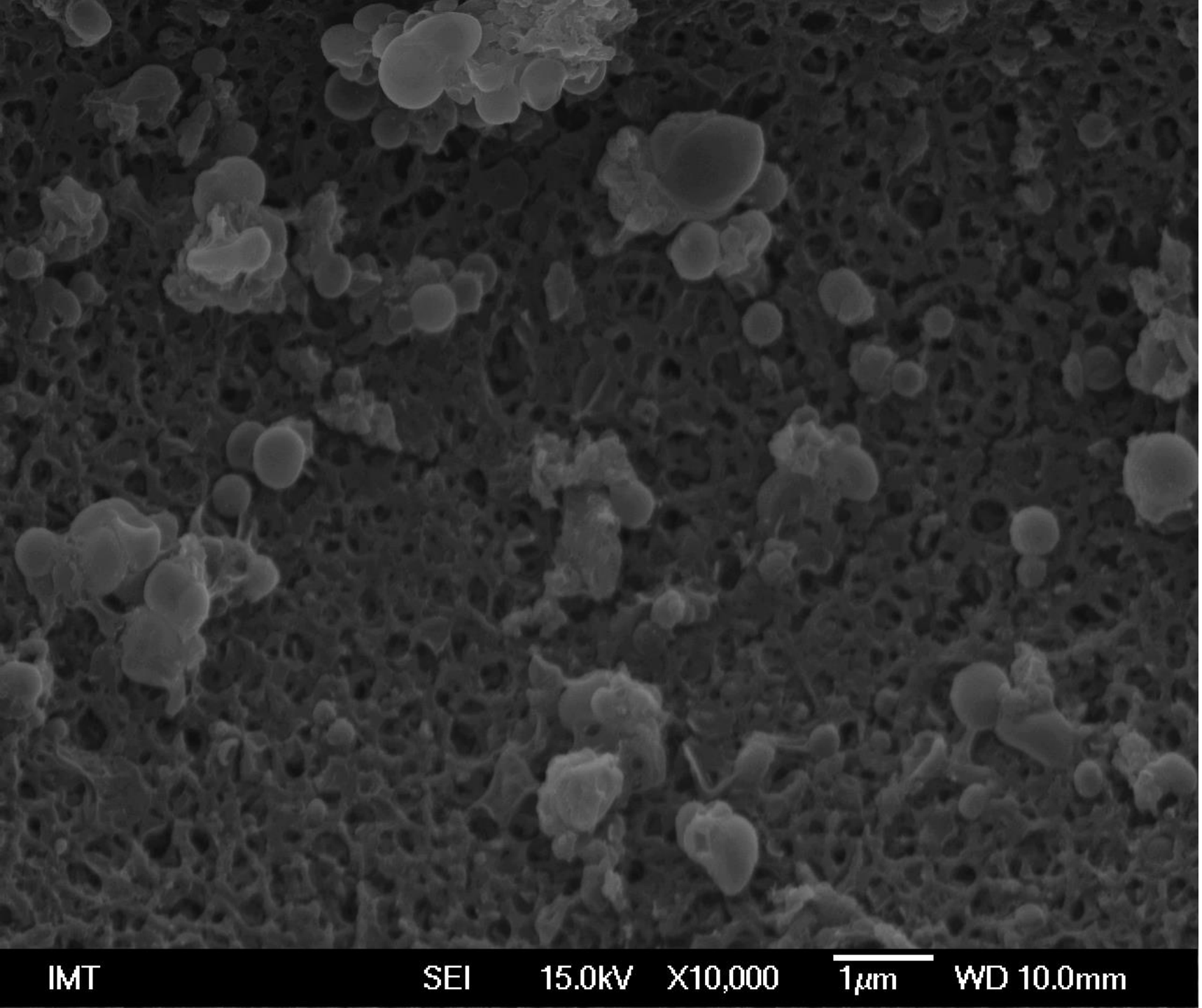

Spruce needle homogenate 10

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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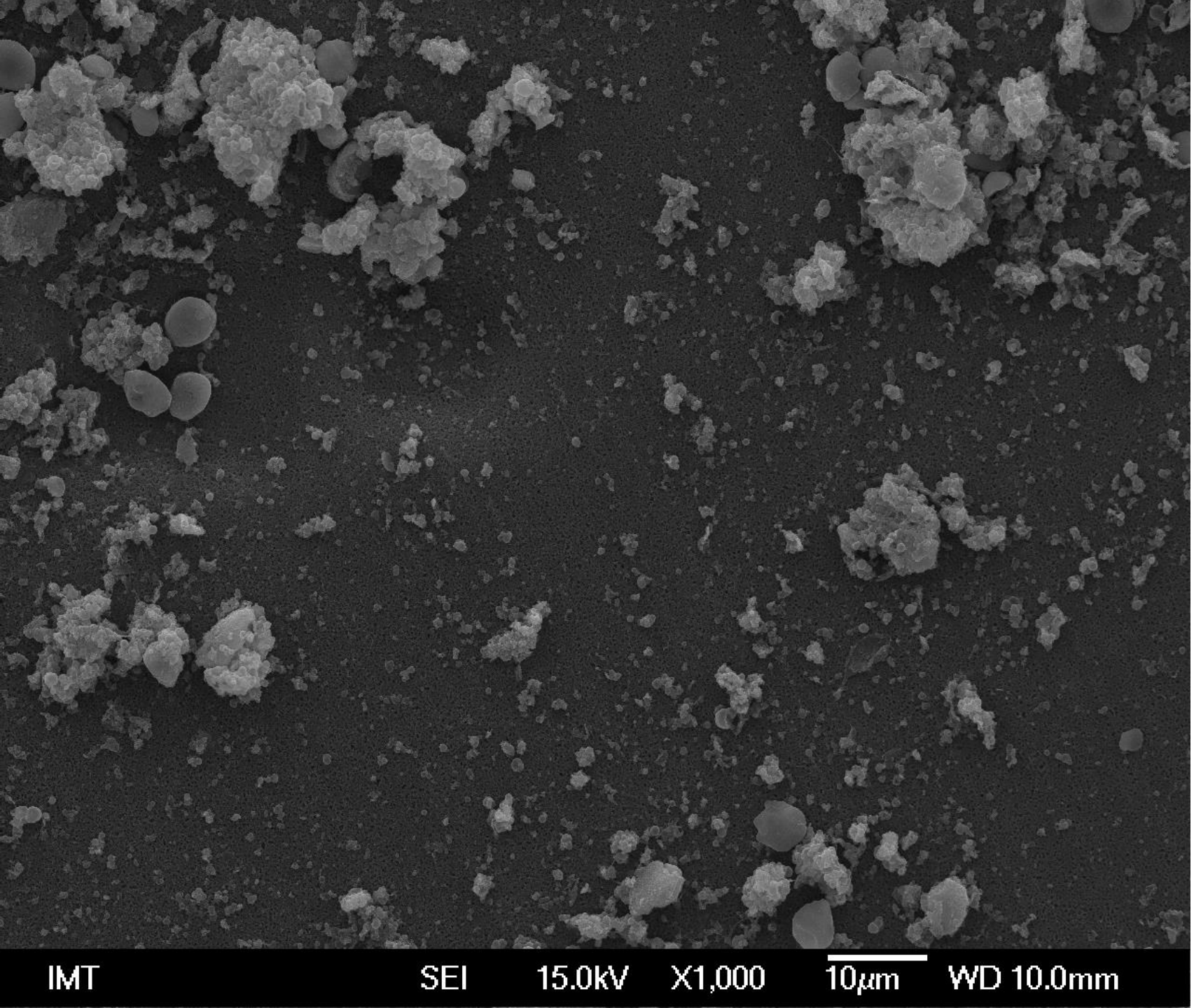

Spruce needle homogenate 11

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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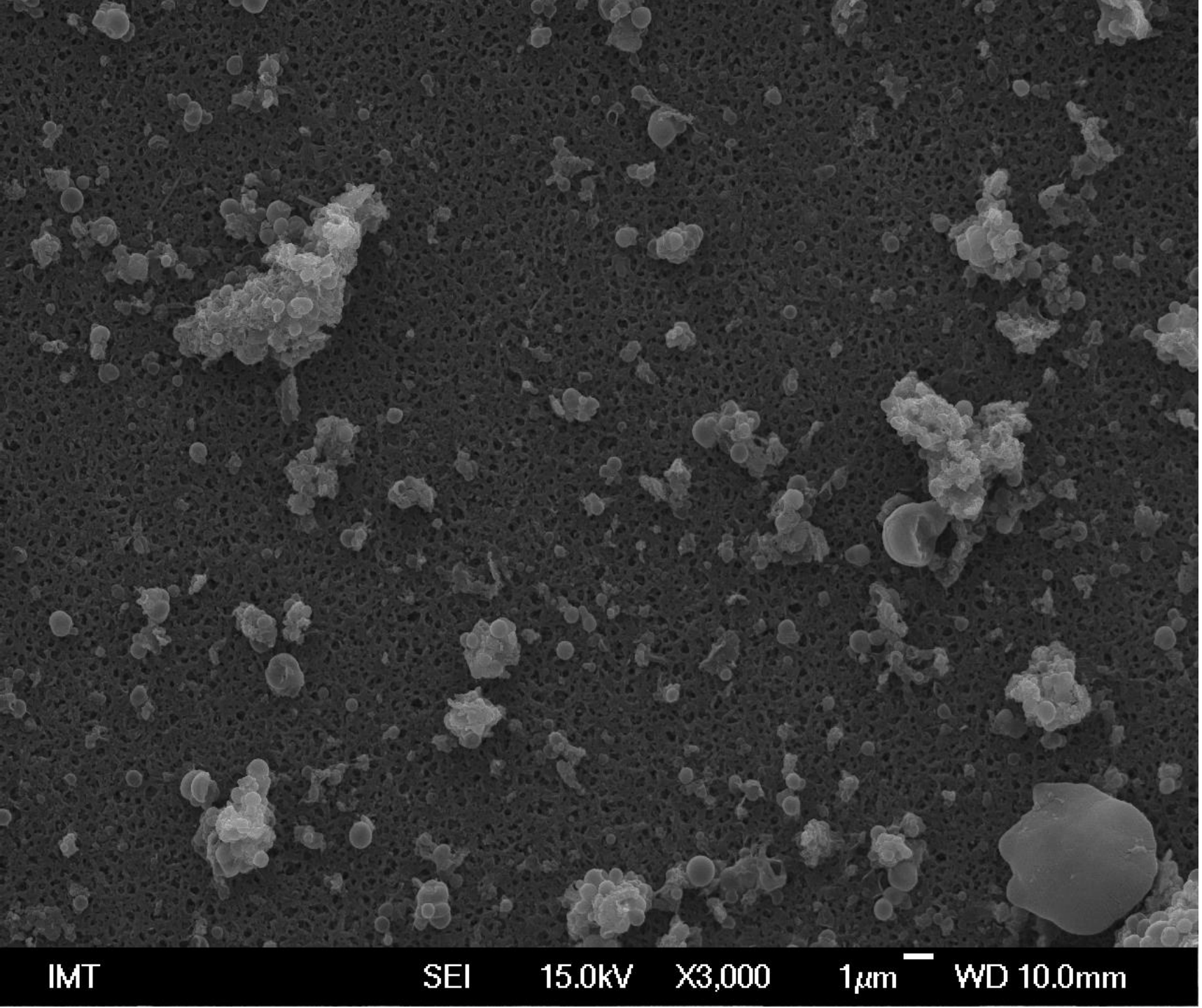

Spruce needle homogenate 12

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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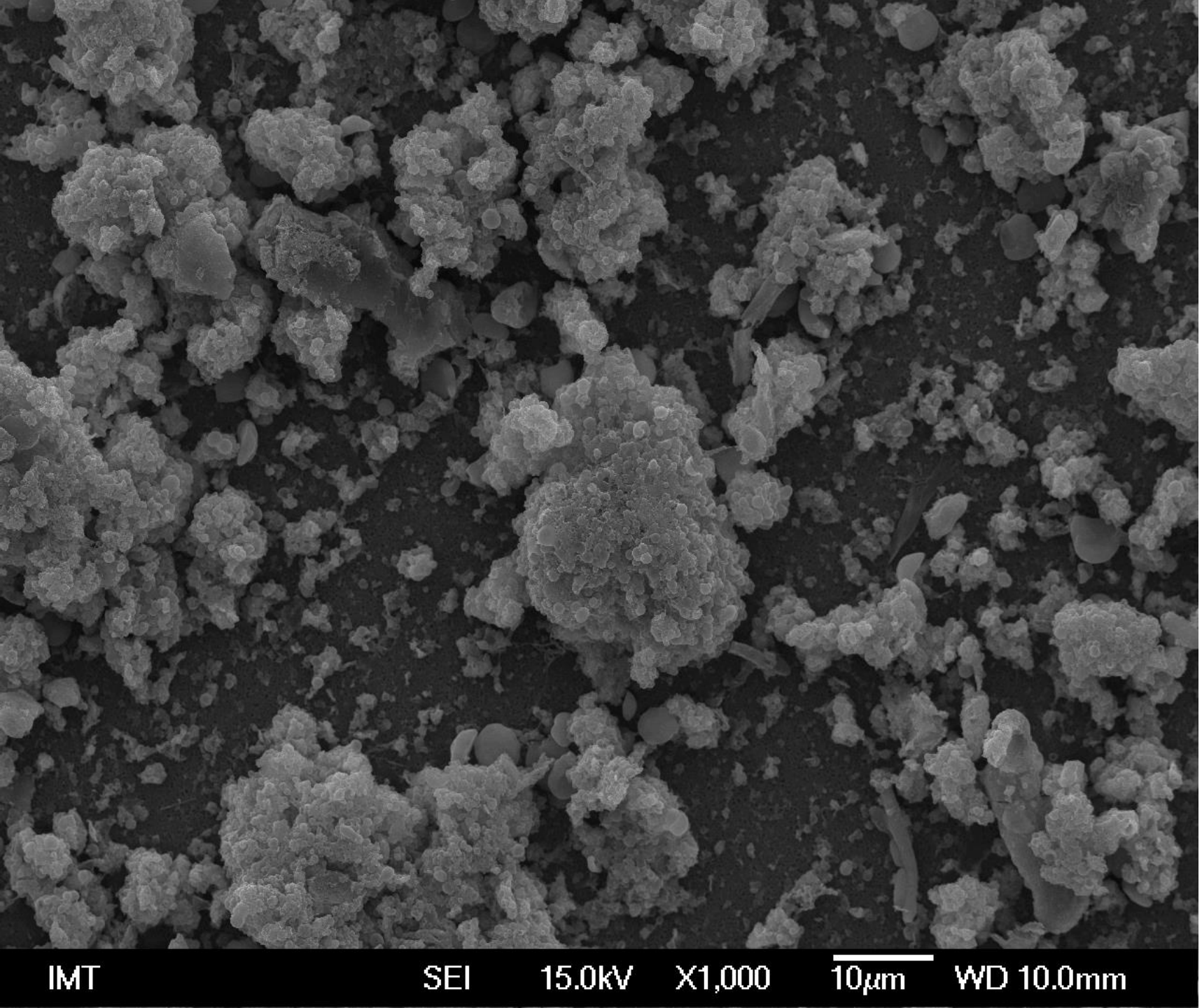

Spruce needle homogenate 13

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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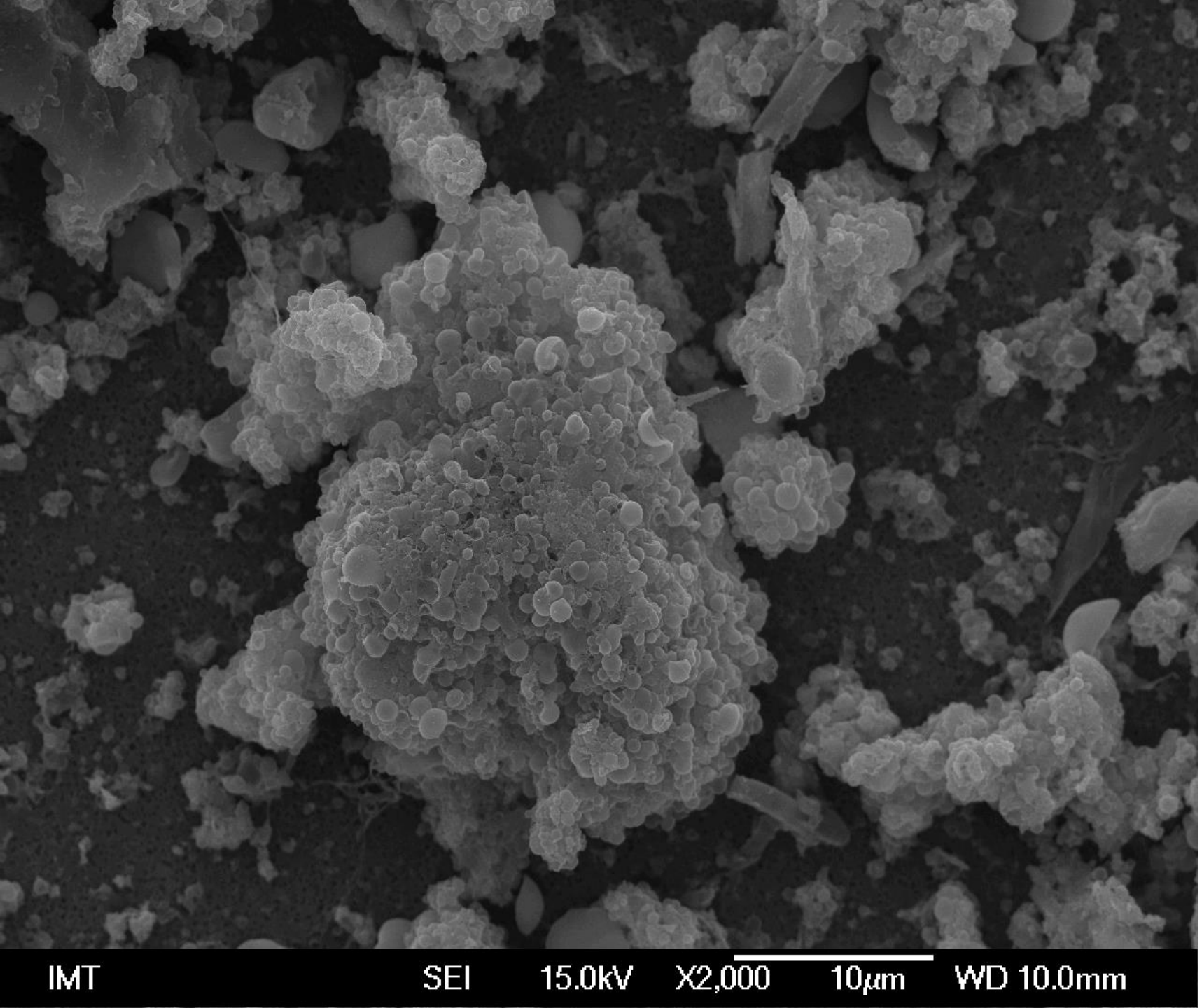
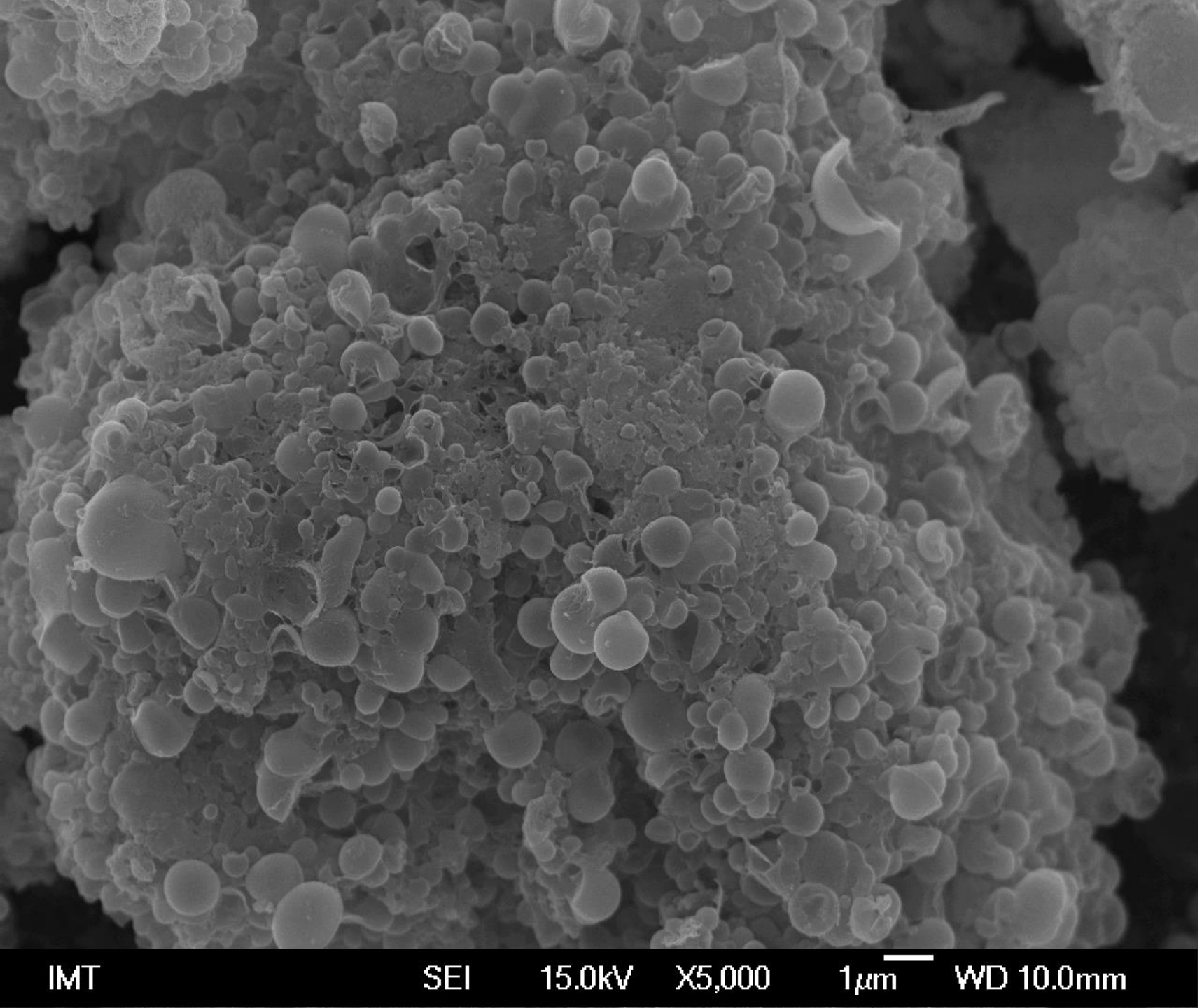


Spruce needle homogenate 14

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

The samples were fixed with OsO4 as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. Int J Mol Sci. 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO4 for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).



Spruce needle homogenate 15

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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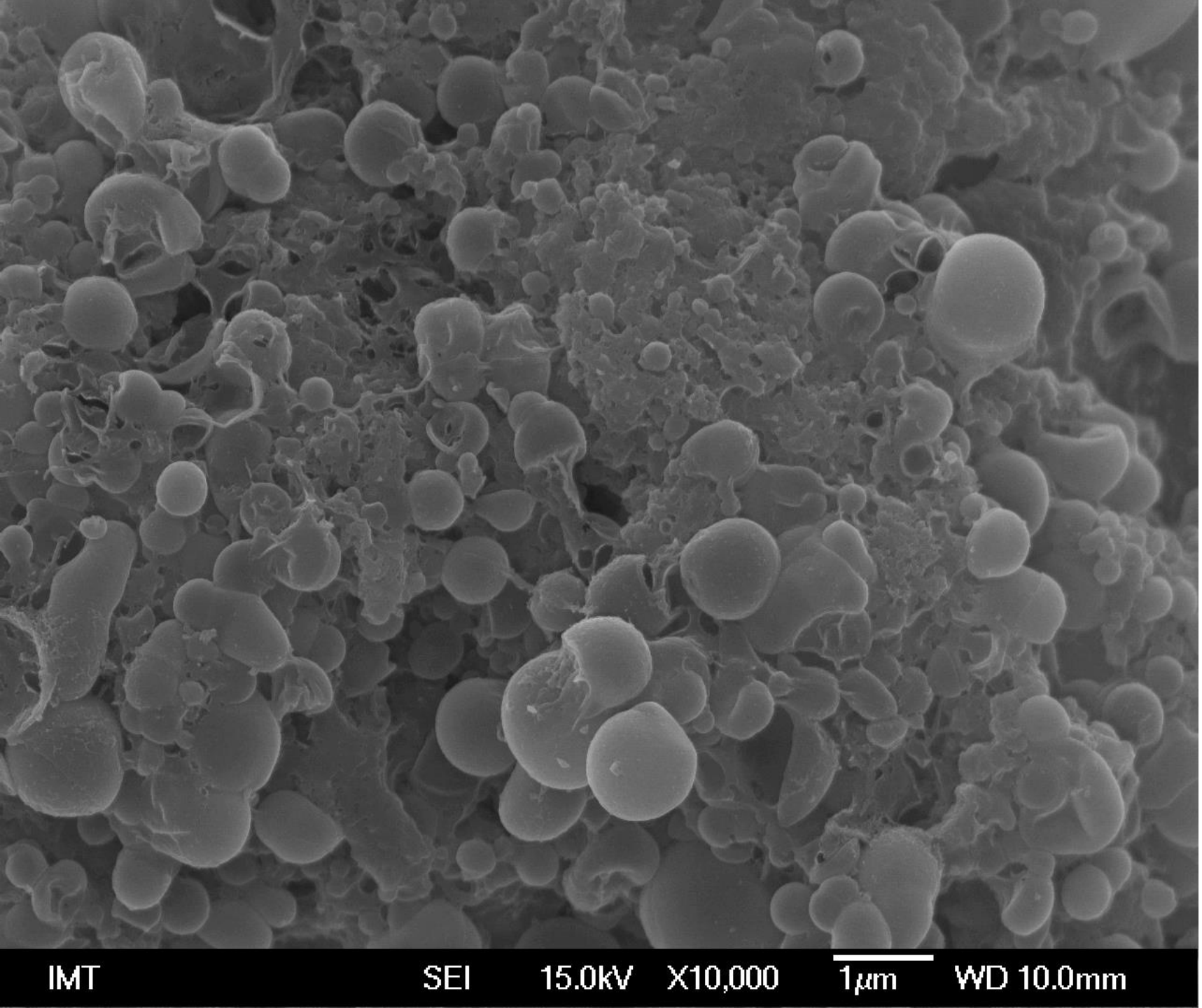

Spruce needle homogenate 16

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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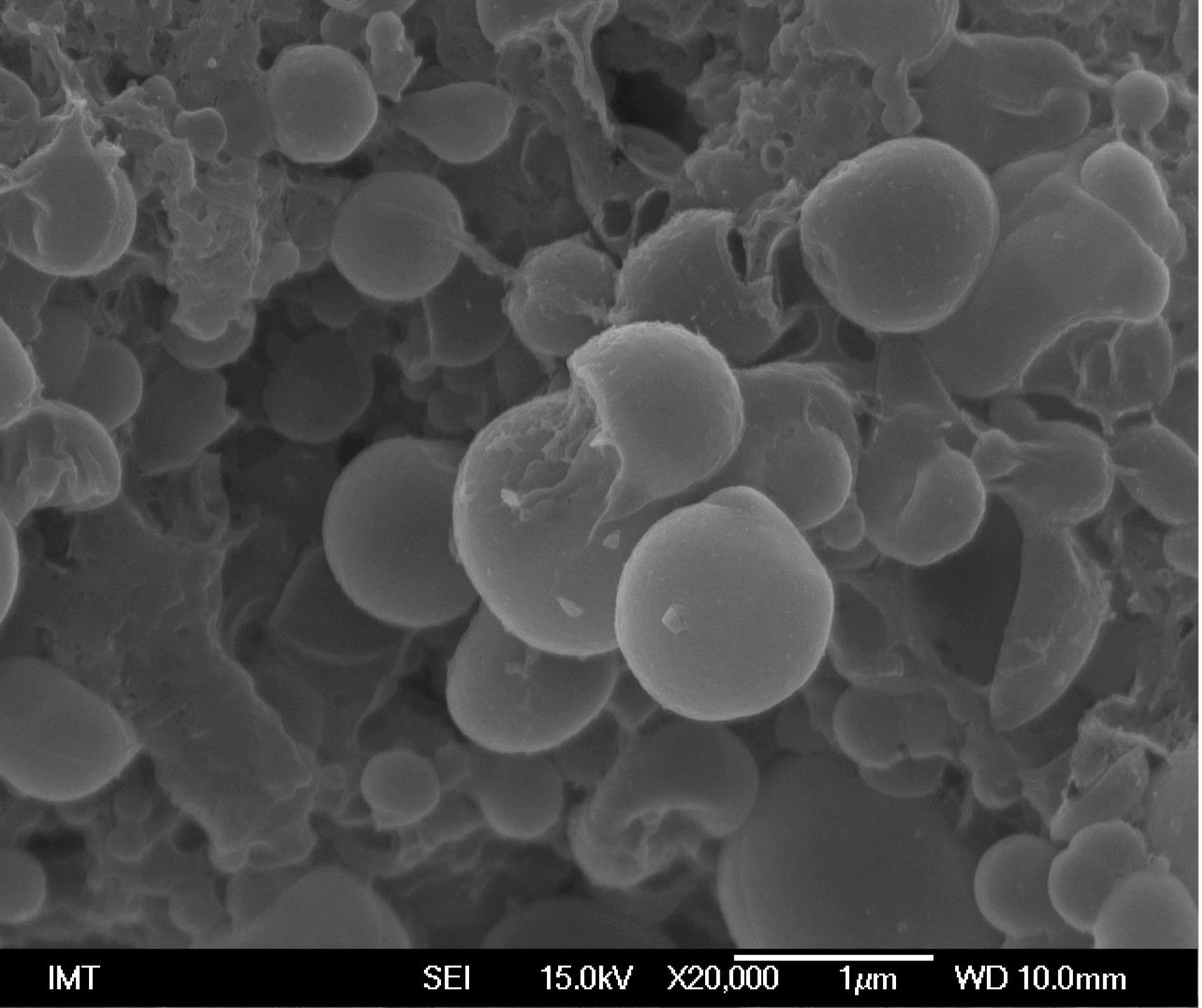

Spruce needle homogenate 17

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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