

1 **Title Page**

2 **Plasticenta: Microplastics in Human Placenta**

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Summary paragraph

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30 **Microplastics are particles smaller than five millimetres obtained from the degradation of**
31 **plastic objects abandoned in the environment. Microplastics can move from the**
32 **environment to living organisms and, in fact, they have been found in fishes and**
33 **mammals.**

34 **Six human placentas, prospectively collected from consenting women with uneventful**
35 **pregnancies, were analyzed by Raman Microspectroscopy to evaluate the presence of**
36 **microparticles. Detected microparticles were characterized in terms of morphology and**
37 **chemical composition.**

38 **12 microparticles, ranging from 5 to 10 μm in size, were found in 4 out of 6 placentas: 5**
39 **in the foetal side, 4 in the maternal side and 3 in the chorioamniotic membranes. All the**
40 **analyzed microparticles were pigmented: three of them were identified as stained**
41 **polypropylene, while for the other nine it was possible to identify only the pigments, which are**
42 **all used for man-made coatings, paints and dyes.**

43 **Here we show, for the first time, the presence of microparticles and microplastics in human**
44 **placenta. This sheds new light on the impact of plastic on human health. Microparticles and**
45 **microplastics in the placenta, together with the endocrine disruptors transported by them,**
46 **could have long-term effects on human health.**

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54 **INTRODUCTION**

55 *“...avoiding the use of plastic and paper, reducing*
56 *water consumption, separating refuse...”*

57 **HOLY FATHER FRANCIS**

58 **From: ENCYCLICAL LETTER LAUDATO SI' ON**
59 **CARE FOR OUR COMMON HOME**

60

61 In the last century, the global production of plastics has grown exponentially by over 350
62 millions of tons per year, and a part ends up polluting the environment¹. It has been estimated
63 that 8.3 billion tons of plastic have been produced since the 1950s, with a constant increase in
64 the last three decades. Global production of plastics currently exceeds 320 million tons (Mt)
65 per year, and over 40% is used as single-use packaging, hence producing plastic waste. In
66 Europe, 26 million tons of plastic waste are produced every year; only 30% is collected for
67 recycling, while the rest is burned or ends up in landfills and it is dispersed into the
68 environment. The degradation that plastics undergo when released into the environment is a
69 serious issue. Exposure to ultraviolet radiation and photo-oxidation in combination with wind,
70 wave action and abrasion, degrade plastic fragments into micro and nanosized particles. These
71 particles pass with relative ease through wastewater filters, making their recovery impossible
72 when they reach the sea. Here, corrosion, high temperatures, waves, wind, ultraviolet radiation,
73 and microbial action, continue the slow process of degradation. The small debris remains at the
74 mercy of the currents, floating, going to the bottom, or ending up on the beaches. In fact, most
75 of the seabed all over the world and in the Mediterranean sea in particular, is made of plastic,
76 resulting from the waste recovered on the coasts and in the sea. Microplastics (MPs) are
77 defined as particles less than 5 mm in size². MPs do not derive only from larger pieces
78 fragmentation, but are also produced in these dimensions for commercial uses. They can be

79 found in aqueous, terrestrial and aerial environments³. Furthermore, there are several reports of
80 microplastics in food⁴, and in particular in seafood, sea salt^{5,6}, and in drinking water⁷.
81 Microplastics have also been detected in the gastrointestinal tract of marine animals^{8,9}.

82 Inside tissues, MPs and microparticles are considered as foreign bodies by the host organism
83 and, as such, trigger local immunoreactions. Furthermore, they can act as carriers for other
84 chemicals, such as environmental pollutants or plastic additives, which are known for their
85 harmful effects^{10,11}.

86 Although there are recent reports highlighting public health concerns due to microplastics
87 presence in food, to date there is little data available. A study reports detection of microplastics
88 in the human intestine¹². There are also reports on microplastics inhalation in humans: this
89 seems to be an important route of diffusion. However, to date microplastics have never been
90 reported within human placentas.

91 In this study, we investigated, for the first time, the presence of microparticles and
92 microplastics in human placentas. Placenta finely regulates foetal to maternal environment
93 and, indirectly, to the external one, acting as a crucial interface via different complex
94 mechanisms¹³. The potential presence of man-made microparticles in this organ may harm the
95 delicate response of differentiation between self and non-self¹⁴, with a series of related
96 consequences that need to be defined.

97 In this light, we performed a Raman Microspectroscopy analysis on digested samples of
98 placenta collected from six consenting patients with uneventful pregnancies, to investigate the
99 presence of microplastics and microparticles.

100

101 **METHODS**

102 Experimental design

103 This was a pilot observational descriptive preclinical study, with prospective and unicentric open
104 cohort. It was approved by the Ethical Committee Lazio 1 (Protocol N. 352/CE Lazio 1; March 31th,

105 2020), and it was carried out in full accordance with ethical principles, including The Code of
106 Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving
107 humans¹⁵. To participate to this study, six selected consenting patients signed an informed consent,
108 which included donation of placentas. The experimental design of the study is sketched in Figure 1.

109 **Enrolment of patients and placentas collection**

110 All recruited women were healthy, at term of pregnancy. Exclusion criteria were:

- 111 • peculiar diets prescribed for any particular medical condition, 4 weeks before delivery;
- 112 • diarrhoea or constipation, two weeks before delivery;
- 113 • antibiotics intake, two weeks before delivery;
- 114 • assumption of drugs affecting intestinal reabsorption (such as activated charcoal, or
115 cholestyramine), two weeks before delivery;
- 116 • diagnosis of gastrointestinal disease (such as ulcerative colitis, or Crohn's disease), cancer,
117 organ transplantation, HIV, or other severe pathologies that needed medical treatment;
- 118 • invasive or abrasive dental treatment, two weeks before delivery;
- 119 • participation to a clinical study, four weeks before delivery
- 120 • alcohol abuse (defined as a >10 score in the Alcohol Use Disorders Identification Test).

121 In order to get information on the chemicals taken by patients the week before delivery, women
122 were asked to fill a questionnaire to record their food consumption (omnivorous, vegetarian, vegan,
123 with no diet restriction), with particular attention to seafood, food sealed in plastic containers/films,
124 beverages in plastic bottles, carbonated drinks, alcoholic drinks, chewing gums containing
125 microplastics. Moreover, patients were asked to take note of the use of toothpastes and cosmetics
126 containing microplastics or synthetic polymers, and cigarette smoking.

127 All six women had a vaginal delivery, at the Department of Obstetrics and Gynaecology of San
128 Giovanni Calibita Fatebenefratelli Hospital, Isola Tiberina, Roma (Italy). All placentas were
129 collected according to a protocol specially designed to be plastic-free, with a special focus on
130 avoiding contaminations from plastic fibres or particles. Obstetricians and midwives used cotton

131 gloves to assist women in labour. In the delivery room, only cotton towels were used to cover
132 patients' beds; graduate bags to estimate postpartum blood loss were not used during delivery, but
133 they were brought in the delivery room only after birth, when umbilical cord was already clamped
134 and cut with metal clippers, avoiding contact with plastic material. After birth, placentas were
135 deposited onto a metal container and immediately taken to the Laboratory of Pathological Anatomy,
136 San Giovanni Calibita Fatebenefratelli Hospital, Isola Tiberina, Roma (Italy). Pathologist,
137 wearing cotton gloves and using metal scalpels, collected from each placenta, portions (mean
138 weight: 23.3 ± 5.7 g) taken from maternal side, foetal side, and chorioamniotic membranes. All
139 samples, strictly anonymous, were labelled with number codes and stored in glass bottles with metal
140 lids at -20°C with no further treatment. By expedited refrigerated transport, samples were shipped
141 to the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences,
142 Università Politecnica delle Marche (Ancona, Italy).

143 **Extraction of microparticles from placenta samples**

144 The extraction of microparticles from the portions of placenta, collected at San Giovanni Calibita
145 Fatebenefratelli Hospital (Rome, Italy) and their analysis by Raman Microspectroscopy were
146 performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental
147 Sciences, Università Politecnica delle Marche (Ancona, Italy). To prevent plastic contamination,
148 cotton laboratory coats, face masks and single-use latex gloves were worn during sample handling,
149 preparation of samples and during the entire experiment. Work surfaces were thoroughly washed
150 with 70% ethanol prior starting all procedures. All liquids (deionised water for cleaning and for
151 preparation of KOH solution) were filtered through $1.6\ \mu\text{m}$ -pore-size filter membrane (Whatman
152 GF/A). Glassware and instruments, including scissors, tweezers and scalpels, were washed using
153 dishwashing liquid, rinsed with deionised water and finally rinsed with $1.6\ \mu\text{m}$ -filtered deionised
154 water. Since the experiments were conducted without the use of the laminar flow hood, the plastic
155 fibres found in the samples were not considered in the results.

156 Microparticles' isolation from placenta samples was performed modifying the protocols from two
157 previous works^{16,17}. Samples were weighed and placed in a glass container cleaned as previously
158 explained. A 10% KOH solution was prepared using 1.6 µm-filtered deionised water and KOH
159 tablets (Sigma-Aldrich). This solution was added to each jar in a ratio with the sample of 1:8 (w/v).
160 The containers were then sealed and incubated at room temperature for 7 days.
161 Digestates were then filtered through 1.6 µm-pore-size filter membrane (Whatman GF/A) using a
162 vacuum pump connected to a filter funnel. The filter papers were dried at room temperature and
163 stored in glass Petri dishes until visual identification and spectroscopic characterization of particles.
164 Three procedural blanks, obtained following the same procedure above described, but without
165 placenta samples and maintained close to the samples during their manipulation, were tested to
166 monitor and correct potential contaminations¹⁶.

167 **Analysis of microparticles by Raman Microspectroscopy**

168 The analysis of microparticles found in the placenta samples was performed by a Raman XploRA
169 Nano Microspectrometer (Horiba Scientific). The following protocol was adopted: (1) to highlight
170 the presence of microparticles (<20 µm), filter membranes were inspected by visible light using a
171 ×10 objective (Olympus MPLAN10x/0.25); (2) the detected microparticles were first
172 morphologically characterized by a ×100 objective (Olympus MPLAN100x/0.90), and (3) then
173 directly analyzed on the filter by Raman Microspectroscopy (spectral range 160-2000 cm⁻¹, 785 nm
174 laser diode, 600 lines per mm grating). The spectra were dispersed onto a 16-bit dynamic range
175 Peltier cooled CCD detector; the spectrometer was calibrated to the 520.7 cm⁻¹ line of silicon prior
176 to spectral acquisition.

177 Raw Raman spectra were submitted to polynomial baseline correction and vector normalization, in
178 order to reduce noise and enhance spectrum quality (Labspec 6 software, Horiba Scientific). The
179 collected Raman spectra were compared with those reported in the SLOPP Library of Microplastics
180 (Spectral Library of Plastic Particles¹⁸) and in the spectral library of the KnowItAll software (Bio-

181 Rad Laboratories, Inc.). Similarities of more than 80 of Hit Quality Index (HQI) were considered
182 satisfactory.

183 All data generated or analysed during this study are included in this published article.

184

185 **RESULTS**

186 From each placenta, three portions (one from maternal side, one from foetal side and one from
187 chorioamniotic membranes, for a total of eighteen pieces) were collected and processed for the
188 subsequent analysis by Raman Microspectroscopy, to verify the presence of microplastics and,
189 more in general, of microparticles similar to man-made products. As described in the Methods
190 section, strict precautions were taken to prevent contaminations; no microparticles were detected on
191 the filters of the blank procedural samples. In total, 12 microparticles, characterized as microplastics
192 and other man-made materials, were detected in the placentas of 4 out of the 6 enrolled patients.
193 In particular, 5 microparticles were found in the foetal side portions, 4 in the maternal side portions,
194 and 3 in the chorioamniotic membranes. All the analyzed microparticles were pigmented; pigments
195 are usually added to polymers in order to colour plastic products, and are added also to coloured
196 paints and coatings, which are ubiquitous as microplastics¹⁹.

197 A retrospective analysis based on Raman spectral information and data reported in literature was
198 performed to define the nature of these microparticles. Firstly, the collected Raman spectra were
199 compared with those stored in the spectral library of the KnowItAll software (Bio-Rad Laboratories,
200 Inc.). Due to the presence of pigments, in many cases, collected Raman spectra resulted mainly due
201 to the signals of the pigment itself^{19,20}. It is known that Raman scattering is more sensitive to the
202 chemical functional groups of pigments, which cover with their signals the entire Raman spectrum,
203 than to the polymeric matrix²¹. In these cases, the KnowItAll software allows to identify the
204 pigments contained in the microparticles. By matching the results from the KnowItAll software
205 with the information obtained by consulting the European Chemical Agency (ECHA²²), it was
206 possible to accurately identify the commercial name, chemical formula, IUPAC name and Color

207 Index Constitution Number of all pigments. Then, in order to uncover the identity of the polymer
 208 matrix of the detected microparticles, the collected Raman spectra were also compared with those
 209 reported in the SLOPP library of microplastics (Spectral Library of Plastic Particles¹⁸). Only for
 210 three microparticles, it was possible to unveil the signals of the polymer matrix in the spectrum.
 211 Table 1 reports the morphological and chemical features of the detected microparticles and relative
 212 pigments, together with information regarding the placenta portion in which they were found.

Table 1. Morphological and chemical features of the detected microparticles and relative pigments, together with information regarding the placenta portion in which they were found (fetal side FS; maternal side MS, and chorio amnio membrane CAM; Hit Quality Index HQI).							
Particle	Placenta Portion	Microparticles					
		Morphology		Polymer matrix	Pigment		
		Size	Color		Generic name	Molecular formula and IUPAC name	HQI
#1	FS	~10 μm	Orange		Iron hydroxide oxide yellow (Pigment Yellow 43; C.I. Constitution 77492)	FeO(OH) iron(III) oxide hydroxide	89.97
#2	CAM	~10 μm	Blue	Polypropylene	Copper phthalocyanine (Pigment Blue 15; C.I. Constitution 74160)	C ₃₂ H ₁₆ CuN ₈ (29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32)copper(II)	82.86
#5	MS	~5 μm	Blue				86.15
#10	FS	~10 μm	Blue				80.60
#3	FS	~10 μm	Blue		Phthalocyanine Blue BN (Pigment Blue 16; C.I. Constitution 74100)	C ₃₂ H ₁₈ N ₈ 29H,31H-phthalocyanine	89.16
#4	MS	~10 μm	Dark blue		Violanthrone (Pigment Blue 65; C.I. Constitution 59800)	C ₃₄ H ₁₆ O ₂ Anthra[9,1,2-cde]benzo[rs]pentaphene-5,10-dione	86.44
#6	MS	~10 μm	Red		Diiron trioxide (Pigment Red 101/102; C.I. Constitution 77491)	Fe ₂ O ₃ Oxo(oxoferriooxy)iron	83.65
#7							89.80
#8	CAM	~5 μm	Dark blue		Pigment Direct Blue 80	C ₃₂ H ₁₄ Cu ₂ N ₄ Na ₄ O ₁₆ S ₄ Dicopper,tetrasodium,3-oxido-4-[[2-oxido-4-[3-oxido-4-[(2-oxido-3,6-disulfonat)naphthalen-1-yl]diazenyl] phenyl]phenyl] diazenyl]naphthalene-2,7-disulfonate	84.55
#9	CAM	~10 μm	Dark blue		Ultramarine Blue (Pigment Blue 29; C.I. Constitution 77007)	Al ₆ Na ₈ O ₂₄ S ₃ Si ₆ Aluminium Sodium orthosilicate trisulfane-1,3-diide	91.96
#11	FS	~10 μm	Violet	Polypropylene	Hostopen violet (Pigment Violet 23; C.I. Constitution 51319)	C ₃₄ H ₂₂ Cl ₂ N ₄ O ₂ 8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo(3,2-b:3'2'-m)triphenodioxazine	80.92
#12	FS	~10 μm	Pink		Novoperm Bordeaux HF3R (Pigment Violet 32; C.I. Constitution 12517)	C ₂₇ H ₂₄ N ₆ O ₇ S 4-[(E)-2-[2,5-dimethoxy-4-(methylsulfamoyl)phenyl]diazen-1-yl]-3-hydroxy-N-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)naphthalene-2-carboxamide	84.57

213 The microphotographs of the analyzed microparticles are reported in Figure 2, together with the
214 collected Raman spectra.

215 The interpretation of the spectral data is discussed below.

216 **Particle #1** (Figure 2a). The collected Raman spectrum resulted perfectly superimposable to the one
217 of the pigment Iron hydroxide oxide yellow: the two spectra shared the main peak at 396 cm^{-1} ,
218 related to the vibrations of iron oxides/hydroxides. This pigment is described as powder or
219 particulate, and it is used for coloration of polymers (plastics and rubber) and in a wide variety of
220 cosmetics, such as BB creams and foundations.

221 **Particles #2 and #10** (Figure 2b). The collected Raman spectra resulted comparable to the one of a
222 polypropylene (PP) blue sample. The Raman spectra of the identified particles shared with the
223 reference spectrum the position of the main peaks, such as the peaks centred at 253 cm^{-1} (wagging
224 of CH_2 moieties, bending of CH moieties), 397 cm^{-1} (wagging of CH_2 moieties, bending of CH
225 moieties), 839 cm^{-1} (rocking of CH_2 and CH_3 moieties, stretching of CC and C- CH_3 moieties), 970
226 cm^{-1} (rocking of CH_3 moieties), and 1455 cm^{-1} (bending of CH_3 and CH_2 moieties), all assigned to
227 PP^{23} . The bands at 679 cm^{-1} , 1143 cm^{-1} , 1340 cm^{-1} and 1527 cm^{-1} , common to reference blue
228 polypropylene and sample spectra, are known to be related to Raman signals of blue pigments,
229 mainly based on copper phthalocyanine^{24,25}.

230 **Particle #3** (Figure 2c). The collected Raman spectrum resulted superimposable to the one of the
231 blue pigment phthalocyanine²⁵. This chemical is reported to be used in adhesives, coating products,
232 plasters, finger paints, polymers and cosmetics and personal care products.

233 **Particle #4** (Figure 2d). The collected Raman spectrum resulted superimposable to the one of the
234 pigment violanthrone. This chemical is used especially for textile (cotton/polyester) dyeing, coating
235 products, adhesives, fragrances and air fresheners. The two main peaks composing both reference
236 and sample spectra are those centred at 1573 cm^{-1} (C-C stretching of benzene ring) and 1307 cm^{-1}

237 (in reference spectrum, an additional shoulder at $\sim 1350\text{ cm}^{-1}$ is visible, assigned to C-C stretching
238 and HCC bending).

239 **Particle #5** (Figure 2e). The collected Raman spectrum resulted perfectly superimposable to the one
240 of the pigment copper phthalocyanine²⁵. Hence, differently from the particle #2 and #10, it was not
241 possible unveiling the identity of the polymer matrix. This pigment is reported to be used for
242 staining of plastic materials, made of polyvinylchloride (PVC), low density polyethylene (LDPE),
243 high density polyethylene (HDPE), polypropylene (PP), polyethylene terephthalate (PET).
244 Furthermore, the pigment 74160 is widely used for staining coating products and finger paints.

245 **Particles #6 and #7** (Figures 2f). The collected Raman spectra resulted perfectly superimposable to
246 the one of the red pigment oxo (oxoferriooxy) iron: the two spectra shared with the reference one
247 the three main peaks at 220, 287 and 401 cm^{-1} , typical of iron oxides²⁶. The same pigment is
248 reported as Pigment Red 101 and 102, depending on its synthetic or natural origin. This pigment is
249 used as food additive, for coloration of plastics, rubber, textiles and paper.

250 **Particle #8** (Figure 2g). The collected Raman spectrum resulted superimposable to the one of the
251 pigment Direct Blue 80. This dye is reported to be used for coloration of leather, paper and textiles.

252 **Particle #9** (Figure 2h). The collected Raman spectrum resulted superimposable to the one of the
253 pigment Ultramarine Blue. This pigment is mainly applied in cosmetics, for example for
254 formulations of soap, lipstick, mascara, eye shadow and other make-up products.

255 **Particle #11** (Figure 2i). The collected Raman spectrum resulted comparable to the one of a PP
256 purple fibre. The Raman spectrum of the identified particle shared with the reference spectrum all
257 the positions of the main peaks, partly ascribable to PP²³ (such as the peaks centred at 397 cm^{-1} ,
258 assigned to the wagging of CH_2 moieties/bending of CH moieties, and at 1455 cm^{-1} , assigned to the
259 bending of CH_3 and CH_2 moieties), but mainly ascribable to the violet pigment (such as the bands
260 centred at 1193 cm^{-1} , 1335 cm^{-1} and 1381 cm^{-1})²⁵.

261 **Particle #12** (Figure 2l). The collected Raman spectrum resulted superimposable to the one of the
262 pink pigment Novoperm Bordeaux HF3R²⁵. The Raman spectrum of this monoazopigment shared

263 with the sample spectrum the main peaks centred at 731 cm⁻¹, 961 cm⁻¹, 1219 cm⁻¹, 1280 cm⁻¹, 1360
264 cm⁻¹, and 1580 cm⁻¹. This pigment is reported to be used to permanently coat and protect wood
265 surfaces, in photographic chemicals, inks and toners, given its high solvent resistance and good heat
266 stability.

267

268

269 **DISCUSSION**

270 In this study, we reported, for the first time, the presence of man-made microparticles and MPs in
271 human placentas. The analysis of portions of maternal side, foetal side and chorioamniotic
272 membranes of human placentas revealed the presence of 12 pigmented microparticles, compatible
273 with microplastics and other man-made materials, in the placentas of 4 women out of the total of the
274 6 analyzed. In particular, 5 microparticles were found in the foetal side, 4 in the maternal side and 3
275 in the chorioamniotic membranes, indicating that these microparticles, once internalized, can
276 colonize placenta tissues at all levels (Figure 3).

277 The identified microparticles were differentiated between stained microplastics (particles #2, #10
278 and #11, all attributable to polypropylene) and paint/coating/dye microparticles, in which the
279 polymer matrix had lower amount (particles #1, #3-9, and #12)¹⁹. All the microparticles were ~10
280 µm in size, except for two that were smaller (~5 µm): these dimensions are compatible with a
281 possible transportation by bloodstream. Previous analyses of 5-10 µm particles, by Electron
282 Microscopy coupled with X-ray microprobe, revealed the presence of microparticles as foreign
283 bodies in human internal organs²⁷.

284 Microparticles and MPs may access the bloodstream and reach placenta from the gastrointestinal
285 tract (GIT)²⁸, from the maternal respiratory system (Figure 4A-B-C-D), or both, by M cells-
286 mediated endocytosis mechanisms, or paracellular transport. It is known that the fraction of inhaled
287 particles, with less than 2.5 µm, is largely retained in the lungs, but can pass through respiratory
288 barriers²⁹. The microparticles isolated in the present study have dimensions of 5-10 µm, making it

289 plausible that they were removed from the respiratory cilia, once internalized by inhalation; in effect,
290 the most probable transport routes for nanoparticles is the diffusion through cellular membranes, while
291 particles with dimensions of 10-20 μm may reach internal organs mostly by mechanisms of particle
292 uptake and translocation, as described for the internalization from the GIT³⁰. GIT persorption is
293 described as the translocation of particles into the circulatory system of the GIT through gaps in the
294 epithelium of the villus tips; it is expected to represent the major uptake route for microparticles.
295 Uptake and subsequent translocation to secondary target organs depend on several factors, including
296 hydrophobicity, surface charge, surface functionalization and the associated protein corona, and
297 particle size. The uptake and translocation to secondary target organs of microparticles were associated
298 with inflammatory responses in the surrounding tissues, such as the immune activation of macrophages
299 and the production of cytokines³¹.

300 Once microparticles have reached the maternal surface of the placenta (Figure 3), they can invade the
301 tissue in depth by several transport mechanisms, both active and passive, that are not clearly
302 understood yet³². The transplacental passage of 5-10 μm size microparticles may depend on the
303 different physiological conditions and genetic characteristics of placenta: this may explain, together
304 with the diverse food habits and lifestyle of patients, the absence of microparticles in 2 of the 6
305 analyzed placentas and the different localization and characteristics of the particles identified in the
306 present study. It is known that a great variability exists in the expression and function of placental
307 drug transporters, both within human populations (interindividual variability) and also during
308 gestation (intraindividual variability)³³. We suppose that this variability exists also in relation to the
309 mechanism of particles' internalization.

310 The presence of microparticles in the placenta tissue requires to reconsider the immunological
311 mechanism of self-tolerance. Placenta represents the interface between the foetus and the
312 environment¹³. Embryos and fetuses must continuously adapt to the maternal environment and,
313 indirectly, to the external one, by a series of complex responses. An important part of this series of
314 responses consists in differentiate self and non-self¹⁴, a mechanism that may be perturbed by the

315 presence of microparticles and MPs. It is in fact reported that, once internalized, MPs may
316 accumulate and exert localized toxicity by inducing and/or enhancing immune responses and,
317 hence, potentially reducing the defence mechanisms against pathogens and altering the utilization of
318 energy stores¹⁰.

319 Potentially, in placenta, MPs, and in general microparticles, may alter several cellular regulating
320 pathways, such as immunity mechanisms during pregnancy, growth-factor signalling during and
321 after implantation, functions of atypical chemokine receptors governing maternal-foetal
322 communication, signalling between the embryo and the uterus, and trafficking of uterine dendritic
323 cells, natural killer cells, T cells and macrophages during normal pregnancy. All these effects may
324 lead to adverse pregnancy outcomes³⁴. Three of the particles identified in the present study (particles
325 #2, #10, and #12) resulted polypropylene (PP). It is known that polymers used in plastic products
326 have cytotoxic effects. For example, the toxicity of PP particles appears related to their size: smaller PP
327 particles may provide more surface area to disturb cell growth. Moreover, it was observed that, when
328 administered as a powder, PP particles, neither smaller nor larger, were cytotoxic, while PP particles
329 dispersed in medium have potentially greater toxicity. The administration of PP particles of dimensions
330 of 5-10 μm resulted in inducing murine macrophage cells to increase IL-6 secretion, suggesting that small
331 PP particles may mimic potential pathogens³⁵.

332 A crucial problem related to microplastics is their potential release of chemicals, which can cause severe
333 damages to cells. In fact, plastic debris has shown to contain various contaminants, including
334 micromolecular substances such as chemicals and monomers. Some of these substances, such as
335 bisphenol A, phthalates and some of the brominated flame retardants, are endocrine disruptors, known
336 to adversely affect human health upon exposure via ingestion and inhalation³⁶. It is reported that low
337 concentrations of bisphenol A can affect cell proliferation in human placental first trimester
338 trophoblasts, downregulating mRNA expression of VEGF and causing an abnormal placental
339 development³⁷. Moreover, phthalates have been found in human urine and blood samples; they are

340 considered responsible of several effects in animals and humans, such as impairment of pubertal
341 development, male and female reproductive health, pregnancy outcomes and respiratory health³⁸.
342 In conclusion, this is the first study revealing the presence of man-made microparticles in human
343 placenta, shedding new light on the level of human exposure to microplastics and microparticles in
344 general. The dimensions of the detected particles are consistent with the known mechanisms of
345 particle uptake and translocation, described for other internalization routes and yet to be clarified in this
346 organ. Due to the crucial role of placenta in hosting the foetus and in acting as an interface between the
347 latter and the external environment, the presence of exogenous and potentially harmful particles is
348 matter of great concern, for the possible consequences on pregnancy outcomes. Further studies need to
349 be performed to increase the number of enrolled patients. Moreover, we are planning to investigate if
350 microparticles are in the intracellular or extracellular compartment of tissues, moving from a digestion-
351 based protocol to a histology-based one. Finally, further analyses will be necessary to assess if the
352 presence of these particles in human placenta may trigger immune responses or determine the release of
353 toxic contaminants, resulting harmful for pregnancy.

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443 **Figure Legends**

444 **Figure 1.** Design of the study.

445 **Figure 2.** Microphotographs and collected Raman spectra of: (a) Particle #1 (scale bar 5 μm); (b) Particles
446 #2 and #10 (scale bar 5 μm for #2 and 10 μm for #10); (c) Particle #3 (scale bar 5 μm); (d) Particle #4 (scale
447 bar 5 μm); (e) Particle #5 (scale bar 5 μm); (f) Particles #6 and #7 (scale bar 10 μm for #6 and 5 μm for #7);
448 (g) Particle #8 (scale bar 10 μm); (h) Particle #9 (scale bar 10 μm); (i) Particle #11 (scale bar 5 μm), and (l)
449 Particle #12 (scale bar 10 μm).

450 **Figure 3.** The figure illustrates the twelve microparticles that we found in the analyzed placentas and
451 described in figure 1. They are located in the placental portion in which they were found.

452 **Figure 4 A-B-C-D. Hypothetical mechanisms by which microplastics penetrate human tissues.**

453 (A) Endocytosis by M cells. At the level of the Peyer's Patch, below the mucous gut, MPs
454 ingested with food can be uptaken by endocytosis from the M cells, transported across the
455 epithelium into the subepithelial dome where they encounter dendritic cells, which in turn transport
456 them through the lymphatic circulation, from where they reach the blood. (B) Paracellular
457 Diffusion. MPs could penetrate through the intestinal lumen from loose junctions. This
458 phenomenon could partially explain why some inflammatory states, which increase loose junctions
459 favour intestinal passage. Once the intestinal lumen has been crossed, the MPs are collected by the
460 dendritic cells and transported in the lymphatic and subsequently in the systemic circulation. (C)
461 Upper airways, At the level of the upper respiratory tract the mucus is thicker and allows a
462 successful clearance of the foreign bodies particles, in addition, the mechanical movement of
463 ciliated epithelium and the presence of surfactant prevents smaller particles from spreading through
464 the epithelium and reach the circulation. (D) Lower airways, In the lower respiratory tract the
465 mucus layer is thinner, thus facilitating the diffusion of particles which, thanks to their particular
466 aerodynamic shape, are able to reach this part of the respiratory tract. Once penetrated, the MPs can
467 spread into the general circulation by cellular uptake or diffusion. (*Modified from: Mowat, A.*
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495 **Acknowledgements** We would like to thank all San Giovanni Calibita Fatebenefratelli Hospital
496 staff for their collaboration during the study and placentas collection.

497 **Author contributions**

498 A.R. and E.G. designed the study; C.S., P.C., V.N., O.C., F.P., M.C.A.R., F.B., S.D., E.D.A. and D.R.
499 performed experiments; A.R., A.S., C.S., P.C., V.N., O.C., F.P., M.C.A.R., F.B., S.D., E.D.A., D.R. and
500 E.G. analysed and interpreted data; A.R., E.G. M.M. and A.S. drafted the manuscript;

501 **Competing interests**

502 The authors declare no competing interests.

503 **Correspondence and requests for materials** should be addressed to A.S.

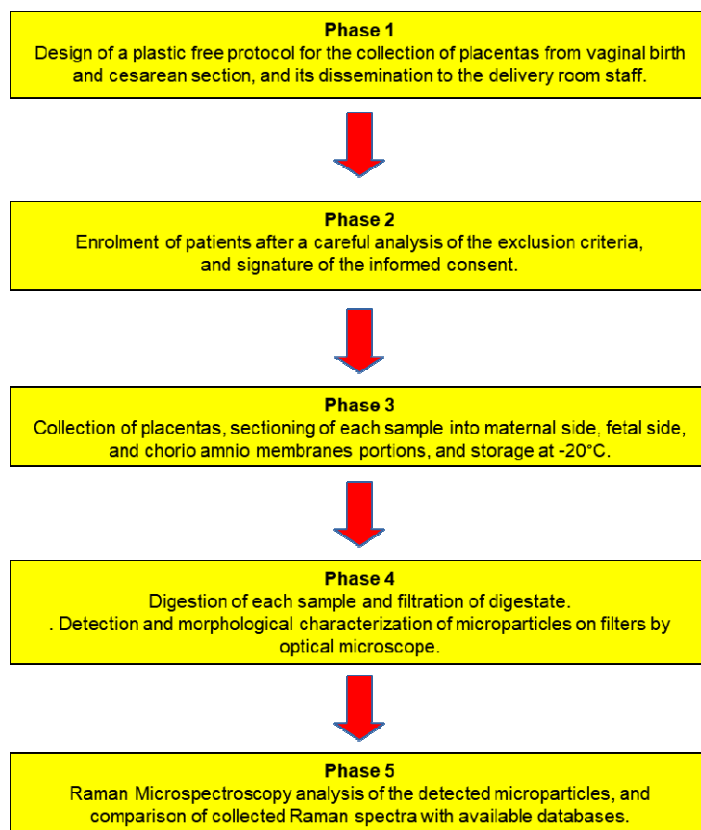


Figure 1. Design of the study.

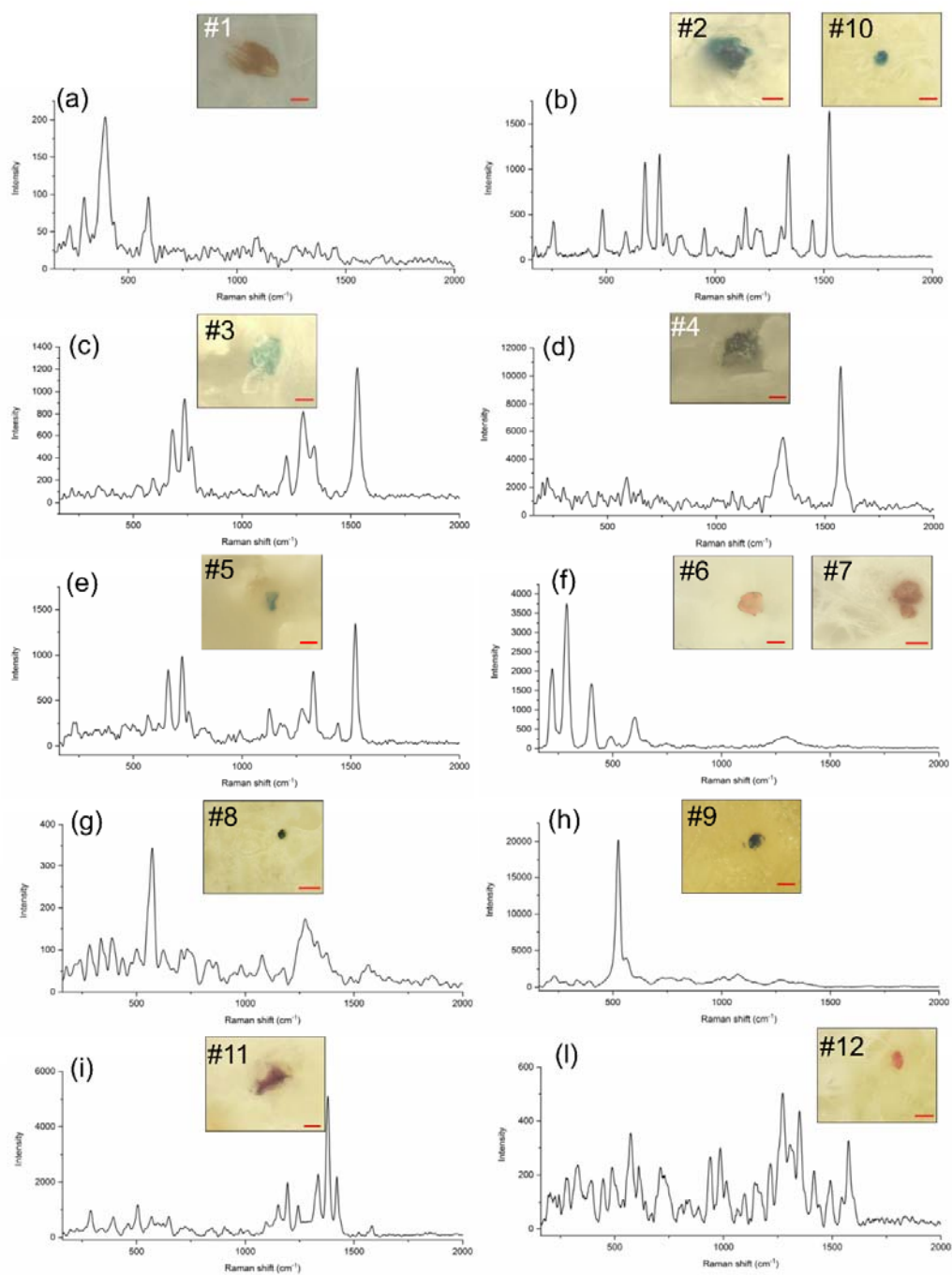


Figure 2. Microphotographs and collected Raman spectra of: (a) Particle #1 (scale bar 5 μm); (b) Particles #2 and #10 (scale bar 5 μm for #2 and 10 μm for #10); (c) Particle #3 (scale bar 5 μm); (d) Particle #4 (scale bar 5 μm); (e) Particle #5 (scale bar 5 μm); (f) Particles #6 and #7 (scale bar 10 μm for #6 and 5 μm for #7); (g) Particle #8 (scale bar 10 μm); (h) Particle #9 (scale bar 10 μm); (i) Particle #11 (scale bar 5 μm), and (l) Particle #12 (scale bar 10 μm).

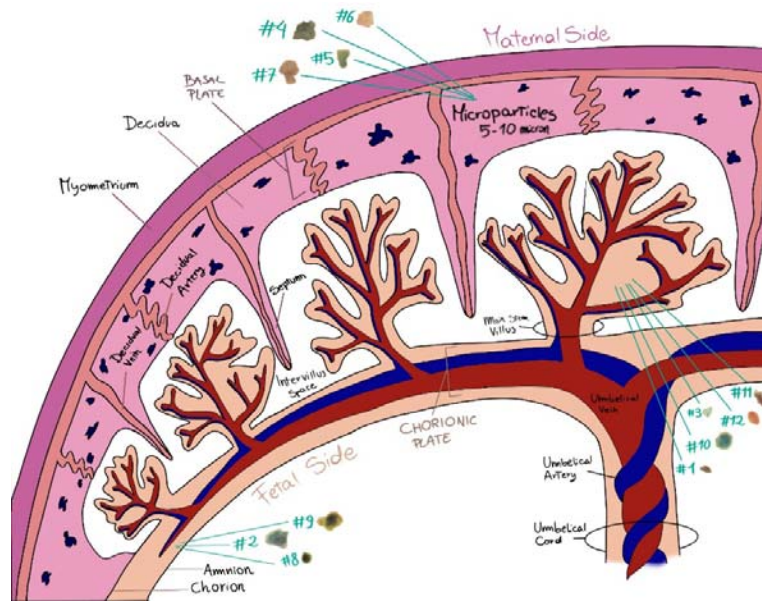


Figure 3. The figure illustrates the twelve microparticles that we found in the analyzed placentas and described in figure 1. They are located in the placental portion in which they were found.

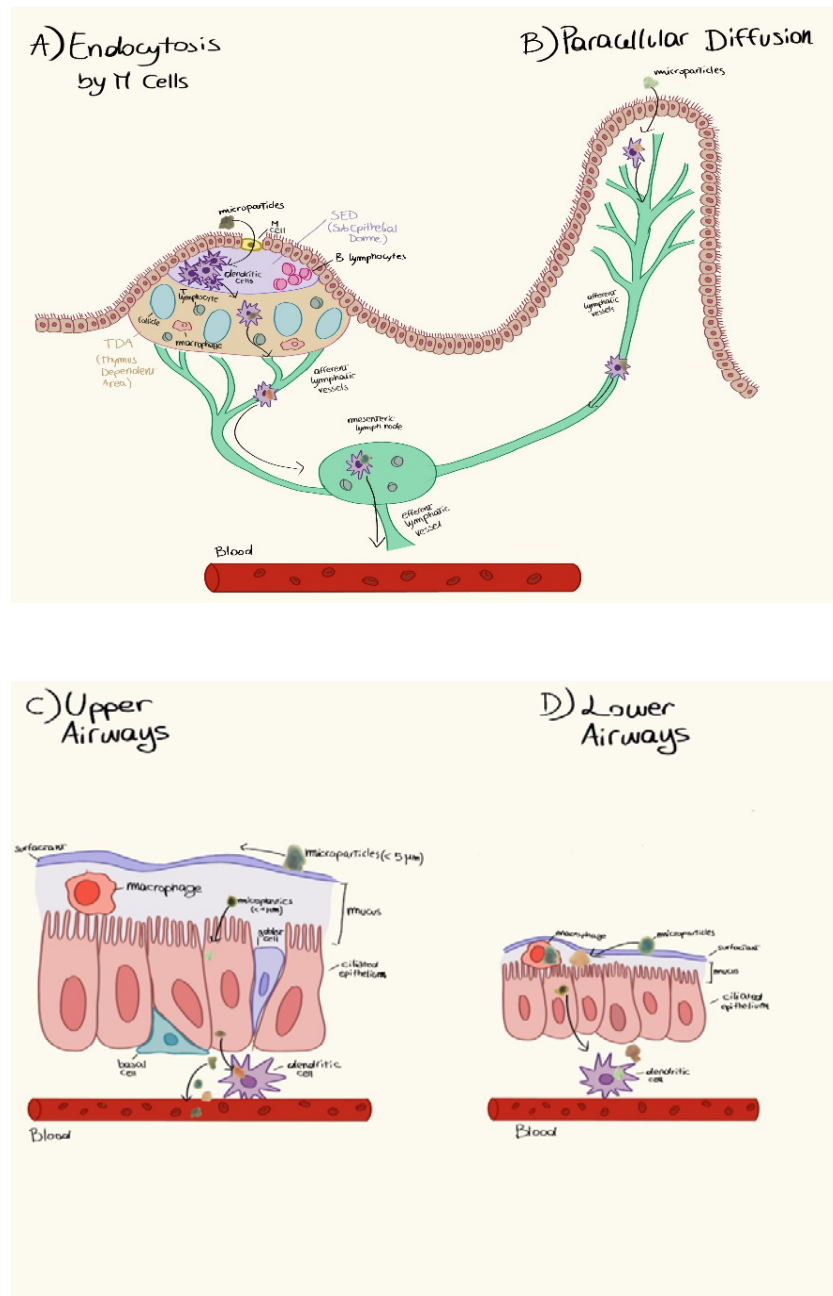


Figure 4 A-B-C-D. Hypothetical mechanisms by which microplastics penetrate human tissues. (A) Endocytosis by M cells. At the level of the Peyer's Patch, below the mucous gut, MPs ingested with food can be uptaken by endocytosis from the M cells, transported across the epithelium into the subepithelial dome where they encounter dendritic cells, which in turn transport them through the lymphatic circulation, from where they reach the blood. (B) Paracellular Diffusion. MPs could penetrate through the intestinal lumen from loose junctions. This phenomenon could partially explain why some inflammatory states, which increase loose junctions favour intestinal passage. Once the intestinal lumen has been crossed, the MPs are collected by the dendritic cells and transported in the lymphatic and subsequently in the systemic circulation. (C) Upper airways, At the level of the upper respiratory tract the mucus is thicker and allows a successful clearance of the foreign bodies particles, in addition, the mechanical movement of ciliated epithelium and the presence of surfactant prevents smaller particles from spreading through the epithelium and reach the circulation. (D) Lower airways, In the lower respiratory tract the

mucus layer is thinner, thus facilitating the diffusion of particles which, thanks to their particular aerodynamic shape, are able to reach this part of the respiratory tract. Once penetrated, the MPs can spread into the general circulation by cellular uptake or diffusion. (*Modified from: Mowat, A. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol 3, 331–341 (2003). <https://doi.org/10.1038/nri1057>. And Ruge, C. A.; Kirch, J.; Lehr, C. M. Pulmonary drug delivery: From generating aerosols to overcoming biological barriers-therapeutic possibilities and technological challenges. Lancet. Respir. Med. 2013, 1(5), 402–413.*)