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Altered formation of the iron oxide nanoparticle-biocorona due to individual variability and exercise

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ABSTRACT

Nanoparticles (NPs), introduced into a biological environment, accumulate a coating of biomolecules or biocorona (BC). Although the BC has toxicological and pharmacological consequences, the effects of inter-individual variability and exercise on NP-BC formation are unknown. We hypothesized that NPs incubated in plasma form distinct BCs between individuals, and exercise causes additional intra-individual alterations. 20 nm iron oxide (Fe_3O_4) NPs were incubated in pre- or post-exercise plasma *ex vivo*, and proteomics was utilized to evaluate BC components. Analysis demonstrated distinct BC formation between individuals, while exercise was found to enhance NP-BC complexity. Abundance differences of NP-BC proteins were determined between individuals and resulting from exercise. Differential human macrophage response was identified due to NP-BC variability. These findings demonstrate that individuals form unique BCs and that exercise influences NP-biomolecule interactions. An understanding of NP-biomolecule interactions is necessary for elucidation of mechanisms responsible for variations in human responses to NP exposures and/or nano-based therapies.

1. Introduction

Nanoparticles (NPs) have the capacity to revolutionize a multitude of technologies across a number of fields, including electronics, consumer products, textiles, biomedicine, and others. Iron oxide (Fe_3O_4) NPs specifically have the ability to be utilized in the field of biomedicine as MRI contrast agents, treatments for anemia, magnetic sensing probes, and drug delivery agents (Babes et al., 1999; Ghazanfari et al., 2016; Jain et al., 2005; Neuberger et al., 2005). When NPs enter biological environments, such as the circulatory system, a coating of biomolecules forms as proteins, lipids, and other compounds adsorb to the NP surface. This coating or biocorona (BC) imparts a new interactive interface thus altering NP physicochemical properties (hydrodynamic size, ζ-potential, and dissolution), resulting in modified NP functionality as well as cellular and biological responses (Clift et al., 2010; Maiorano et al., 2010; Monopoli et al., 2012; Shannahan et al., 2015b; Walczyk et al., 2010). BC-induced variations in cellular interactions will likely impact the use of NPs for biomedical applications by modifying biodistribution, clearance, immune response, and toxicity (Kreuter, 2013; Mornet et al., 2004; Tenzer et al., 2013). Specifically, addition of the BC to superparamagnetic Fe_3O_4 NPs decreases their effectiveness as MRI contrast agents and drug delivery vehicles (Amiri et al., 2013; Gupta and Gupta, 2005). In order to fully utilize NP-based therapeutics, it is necessary to first understand these initial NP-biomolecule interactions and their biological implications.

The formation of the NP-BC is governed by NP physicochemical properties, time, and the biological environment. To date, the majority of investigations have examined specific NP properties (composition, charge, size, surface coating, defects, etc.) and their role in NP-BC formation (Jedlovszky-Hajdu et al., 2012; Lundqvist et al., 2008; Monopoli et al., 2011; Raghavendra et al., 2017; Walkey and Chan, 2012). Few studies, however, have evaluated the impact of variations in the biological environment (Raghavendra et al., 2017; Shannahan, 2017). Due to their many biomedical applications, NPs will be utilized in highly variable biological environments, which may impact their functionality. Specifically, individual variations in the biomolecular composition within the circulation are of concern for Fe_3O_4 NPs. Numerous conditions and factors can contribute to variability in circulating macromolecules, including underlying disease state, gender, diet, and others (Gordon et al., 1977; Jenkins et al., 1993; Murphy, 2014;

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Tran et al., 1983). Exercise, which is one of the focuses of our current study, is known to alter circulating biomolecules differently based on the type, duration, and intensity of the exercise (Balfoussia et al., 2014; Dreyer et al., 2006; Kraus et al., 2002; Tran et al., 1983). Ultimately, the variability of biological environments present within our population, as well as activity-induced alterations in circulating biomolecules, could modify BC formation and result in differential biological interactions.

In our current study, we examined differences in the formation of the Fe_3O_4 NP-BC due to inter-individual variations as well as exercise-induced intra-individual variability by utilizing human plasma samples. Further, a human macrophage cell line was utilized to assess the toxicological consequences (cellular association, cytotoxicity, and inflammatory activation) of distinct Fe_3O_4 NP-BCs. In order to perform this investigation, 10 human subjects who did not exercise regularly were recruited and blood samples were collected prior to and following a 7-day exercise regimen. Overall, this descriptive study was designed to demonstrate potential differences between individuals' NP-BCs, as well as the influence of exercise. By elucidating possible variability in the formation of the NP-BC and consequential alterations in immune cell interactions, individual susceptibility to NP-induced health effects may be identified and mitigated.

2. Materials and methods

2.1. Blood collection

Fasting plasma samples were isolated from blood extracted from human subjects (six male, four female) between the ages of 21–32 y who, according to a self-survey, did not smoke or exercise regularly (Table 1). Subjects were exercised once a day at 70% of maximum for 45 min on a stationary exercise bicycle for 7 consecutive days. On days 2, 4, and 6, subjects also performed 3 sets (8–12 repetitions at 80% of maximum) of leg press resistance exercise. Subjects were instructed to not modify any additional aspects of their lifestyle, including diet, for the duration of the study. Fasting blood was again collected from each subject on day 8 at least 12–14 hours after the final exercise session. After each collection, blood was treated with heparin to prevent clotting. The Biomedical Institutional Review Board at Purdue University approved the study protocol, recruitment materials, and consent forms. All study participants gave informed consent and received monetary compensation for their participation.

2.2. Plasma sample characterization

An aliquot of each blood sample was analyzed by Mid America Laboratories to determine glucose, insulin, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol levels. Subjects were ordered 1–10 based on total cholesterol content, with 1 having the lowest total pre-exercise cholesterol and 10 having the highest. Each subject retained the same number pre- to post-exercise. Plasma was isolated from another aliquot of blood by centrifugation at 1300 rcf for 15 min at 4 °C and stored at -80 °C. Plasma samples from the 10 subjects collected prior to exercise were used to determine inter-individual differences in NP-BC formation. Plasma samples collected from 6 subjects post-exercise were used to evaluate exercise-induced differences in BC formation. This was accomplished by comparing the NP-BC produced following incubation in post-exercise plasma to that produced from pre-exercise plasma from the same human subject. These 6 post-exercise plasma samples were selected based on alterations in triglyceride levels following exercise. Specifically, 3 subjects demonstrated minor alterations in triglyceride levels following exercise, whereas 3 other subjects exhibited greater alterations in triglycerides levels (Table 1).

Table 1
Subject Blood Parameters Pre- and Post-Exercise.

A. Pre-Exercise Blood Results

Subject #	Sex	Age	BMI	Glucose (mg/dl)	Insulin (uIU/ml)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total Cholesterol (mg/dl)
1	M	27	24.5	86	5	65	40	90	143
2	M	21	30.8	99	12	128	38	90	154
3	M	21	23.3	96	11	88	37	101	156
4	F	29	24.1	90	8	63	56	100	169
5	F	27	33.7	82	52	176	42	101	178
6	F	30	21.8	92	9	90	52	118	188
7	M	24	22.0	81	9	143	59	105	193
8	M	32	25.9	96	8	111	34	161	217
9	F	21	23.3	75	12	131	54	152	232
10	M	32	34.1	89	27	194	33	196	268

B. Post-Exercise Blood Results

Subject #	Sex	Age	BMI	Glucose (mg/dl)	Insulin (uIU/ml)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total Cholesterol (mg/dl)
1*	**M**	**27**	**24.5**	**93**	**5**	**54**	**44**	**86**	**141**
2*	**M**	**21**	**30.8**	**92**	**5**	**65**	**48**	**118**	**179**
3	M	21	23.3	96	11	62	36	80	128
4	F	29	24.1	94	7	46	61	96	166
5*	**F**	**27**	**33.4**	**92**	**12**	**77**	**45**	**109**	**162**
6*	**F**	**30**	**21.8**	**87**	**11**	**80**	**49**	**84**	**149**
7*	**M**	**24**	**22.0**	**84**	**7**	**76**	**51**	**88**	**154**
8*	**M**	**32**	**26**	**96**	**7**	**104**	**36**	**151**	**208**
9	F	21	23.5	84	10	107	58	135	214
10	M	32	33	87	25	152	29	210	269

After subjects' blood was drawn, aliquots were analyzed by Mid America Laboratories. A. Blood parameters prior to completion of 7-day exercise regimen. B. Blood parameters following completion of 7-day exercise regimen. * and **bold** indicate subjects selected for further analysis due to large (2, 5, 7) or small (1, 6, 8) change in triglycerides pre- to post-exercise.