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Research Article
Quantitative Analysis of Opsonisation-Enhanced Phagocytosis Across Diverse Pathogens

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Abstract

Background and Objective: Phagocytosis remains a pivotal component of the immune defence mechanism, orchestrated by professional phagocytes, including macrophages and neutrophils. This rigorous investigation delineates the influence of human serum and opsonisation on the phagocytic uptake of three distinct microorganisms. **Materials and Methods:** The study compared phagocytosis efficiency with and without serum using *E. coli*, *S. aureus* and *S. cerevisiae*. Microorganisms were pre-treated with human serum for opsonisation and incubated with macrophage-like cells. ImageJ quantified phagocytic uptake and the Phagocytic Index (PI) was calculated. Statistical analysis using Student's t-test confirmed significantly higher uptake in opsonised samples. A p-value of less than 0.05 was considered statistically significant. **Results:** Quantitative analysis revealed a notable escalation in the phagocytic index (PI) across examined pathogens: The PI for *S. cerevisiae* surged from 0.086 to 1.043, paralleling a percentage increase from 5.64 to 35.75%, whereas *S. aureus* demonstrated an ascent from 0.344 to 2.980, corresponding to a rise in phagocytic percentage from 14.06 to 54.93%. **Conclusion:** This study substantiates the premise that opsonisation fundamentally enhances phagocytic efficiency, positing potential therapeutic innovations to bolster immune responses, particularly in immunocompromised cohorts.

Key words: Phagocytosis, opsonisation, human serum, immune response, phagocytic index, pathogen clearance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phagocytosis is an important type of immune defence mechanism. This term currently usually applies to the ability of professional phagocytes in the immune system to recognize, engulf and eliminate foreign antigens, particularly bacteria, through their phagocytic receptors. Professional phagocytic cells include polymorphonuclear neutrophils, professional monocytes, dendritic cells and tissue-resident macrophages in several organs. Therefore, phagocytosis is important in the clearance of bacteria and cell debris, antigen presentation to lymphocytes, production of antimicrobial agents and immune regulation. It has long been recognized that phagocytosis can be regulated by a series of factors, such as temperature, pH and ion concentrations; phagocytic cells; and the substrates[1].

Understanding host-pathogen interaction is a key element of better understanding the human immune system. On the one hand, insights from this investigation could contribute to therapeutic approaches that target or enhance phagocytosis. For instance, developing treatments that optimize opsonisation could improve pathogen clearance in immunocompromised patients (e.g., suffering from HIV) or during infections with particularly resistant organisms. On the other hand, studying pathogens and host-pathogen interactions could help us better understand microorganisms and our biology. In this study, three microorganisms were selected to investigate immune defence mechanisms: *Escherichia coli* (gram-negative bacteria), *Staphylococcus aureus* (gram-positive bacteria) and Saccharomyces cerevisiae (yeast).

For centuries, pathogens adjusted their virulence to better evade host cells and immune system cells like macrophages. Pathogens like *Staphylococcus aureus*, after entering the host cell, can inhibit apoptosis by upregulating anti-apoptotic genes, such as BCL2 and MCL1. Also, *S. aureus* toxins may prevent the release of cytochrome C from the mitochondrial organelles. This prevents the programmed cell death of the infected macrophage[2].

Another pathogen that is particularly troublesome for macrophages is *Mycobacterium tuberculosis* (Mtb). This pathogen can interfere with the normal maturation of the phagosome, preventing its fusion with lysosomes. This inhibits the activation of hydrolytic enzymes and simultaneously prevents the degradation of the pathogen[3].

To maintain phagocytic function, cells like neutrophils and macrophages need to keep their cytoplasmic pH in check[4]. Both *S. aureus* and Mtb have developed strategies to evade the regulation of phagosome pH in macrophages. Mtb prevents phagosome acidification by disrupting the recruitment of V-ATPases, maintaining a neutral pH that inhibits the activation of destructive enzymes. Mechanisms of *S. aureus* are less known than Mtb and future research is necessary[5].

The host defences adjust their pathogen-associated molecular patterns (PAMPs) because of the different structures of gram-positive and gram-negative bacteria. Gram-negative bacteria (*E. coli*) have lipopolysaccharides (LPS) that interact differently with macrophages compared to Gram-positive bacteria (*S. aureus*), which have thicker peptidoglycan layers. The LPS on the outer membrane binds to Toll-like receptor 4 (TLR4) on macrophages, leading to the activation of strong pro-inflammatory signalling pathways, such as the NF-κB pathway, resulting in the production of cytokines like TNF-α, IL-6 and IL-1β[6]. Gram-positive bacteria are more sophisticated and usually hide inside host cells, so they don't trigger inflammation. Apart from that, a thick peptidoglycan layer and teichoic acids of gram-positive bacteria can still be recognised by Toll-like receptor 2 (TLR2) on the cell surface of macrophages[7].

Unlike bacteria, yeast cells (*S. cerevisiae*) have a more complex cell wall structure composed of polysaccharides like glucans, mannans and chitin. The PAMPs recognise those yeast components in macrophages, leading to the release of both pro-inflammatory and anti-inflammatory cytokines[8,9].

This study aims to investigate the mechanisms underlying microbial uptake by phagocytic cells, the role of serum in this process and the significance of opsonisation. Opsonisation, a process that marks pathogens, enhances their uptake by phagocytic cells such as macrophages[10]. Furthermore, serum is known to possess significant antipathogenic properties due to its abundance of antibodies and complement proteins, which facilitate opsonisation of phagocytes. Consequently, it can be hypothesized that the microorganisms *Escherichia coli*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* will exhibit enhanced phagocytosis in serum-containing samples[8]. Additionally, this study examines the differences in phagocytosis between gram-negative and gram-positive bacteria, as well as the potential variations in immune response between bacteria and yeast.

MATERIALS AND METHODS

Study area: The study was conducted at Brunel University London, United Kingdom, in the Department of Life Sciences during the academic year 2024-2025. All experimental procedures were carried out in the University's Biomedical Research Laboratories, under controlled laboratory conditions[5].

Study materials: Three microorganisms were used in this study: *Escherichia coli* (gram-negative bacteria), Staphylococcus aureus (gram-positive bacteria) and *Saccharomyces cerevisiae* (yeast). All microbial strains were obtained from the Brunel University teaching and research culture collection. Human serum was used as the opsonising agent, collected from healthy volunteers under appropriate laboratory safety and ethical guidelines. Phagocytic uptake was assessed using prepared slides of human macrophage-like cells co-incubated with the microorganisms.

Study design: The experiment was designed to compare phagocytosis efficiency in the presence and absence of serum (opsonisation). For each microorganism, duplicate sets of samples were prepared: (i) Non-opsonised microorganisms incubated with host phagocytes and (ii) opsonised microorganisms pre-treated with human serum before incubation with phagocytes[1]. For each condition, ten representative microscopic images were acquired and analysed using ImageJ software.

Measured parameters: The following parameters were recorded for each sample:

- Total number of phagocytes per field
- Number of phagocytes containing internalised microorganisms (positive cells)
- Total number of microorganisms phagocytosed per positive cell
- Phagocytic Index (PI), calculated as:
- PI = Number of microorganisms ingested/Total number of phagocytes observed

These values were used to quantify differences in phagocytic activity between opsonised and non-opsonised groups for each microorganism[4].

Statistical analysis: All data were recorded in Microsoft Excel and further analysed using GraphPad Prism (version X). Results are expressed as Mean±Standard deviation (SD). Differences between opsonised and non-opsonised groups were assessed using Student's t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Phagocytosis activity was markedly enhanced in opsonised samples compared to non-opsonised controls (Table 1). The phagocytic index (PI) of non-opsonised cells significantly higher PI values between 1.043 and 2.980. Correspondingly, the percentage of phagocytosis increased from 5.64-21.83% in non-opsonised cells to 35.75-54.93% in opsonised cells. The mean number of microbes per positive cell also reflected this trend, rising from 1.533-2.444 in non-opsonised samples to 2.919-5.425 in opsonised samples, indicating greater microbial uptake upon opsonisation.

Statistical analysis confirmed that opsonisation significantly improved phagocytosis efficiency across all tested organisms (Table 2). The t-values for *S. cerevisiae*, *E. coli* and *S. aureus* were 3.675, 3.434 and 4.746, respectively, exceeding the critical P value of 2.262 ($p<t1,2,3$). This demonstrates that opsonised cells exhibited significantly higher phagocytic activity than non-opsonised cells.

Visual examination using fluorescence microscopy corroborated these quantitative results (Fig. 1). Non-opsonised macrophages (Fig. 1(a-e) displayed limited uptake of *S. cerevisiae*, *E. coli* and *S. aureus*, with only a few microbes observed within macrophages. In contrast, opsonised cells (Fig. 1b,d,f) showed pronounced microbial internalisation, indicated by multiple green fluorescent microbes within the blue-stained macrophage nuclei. This confirmed that opsonisation markedly enhances the phagocytic capacity of macrophages.

The data analysed in this paper were statistically significant ($p < 0.05$), indicating that the observed differences in phagocytosis between non-opsonized and opsonized samples are statistically reliable and unlikely to have occurred by chance (Table 2).

Table 1: Comparison of phagocytic activity and mean microbial uptake in opsonised and non-opsonised samples

Sample	PI	Phagocytosis (%)	Mean number of microbes per positive cell
1	0.086	5.64	1.533
2	1.043	35.75	2.919
3	0.507	21.83	2.320
4	1.303	37.98	3.430
5	0.344	14.06	2.444
6	2.980	54.93	5.425
Legend		Non-opsonized	Opsonized

Organism in sample: 1-2 Saccharomyces cerevisiae, 3-4 *Escherichia coli*, 5-6 Staphylococcus aureus

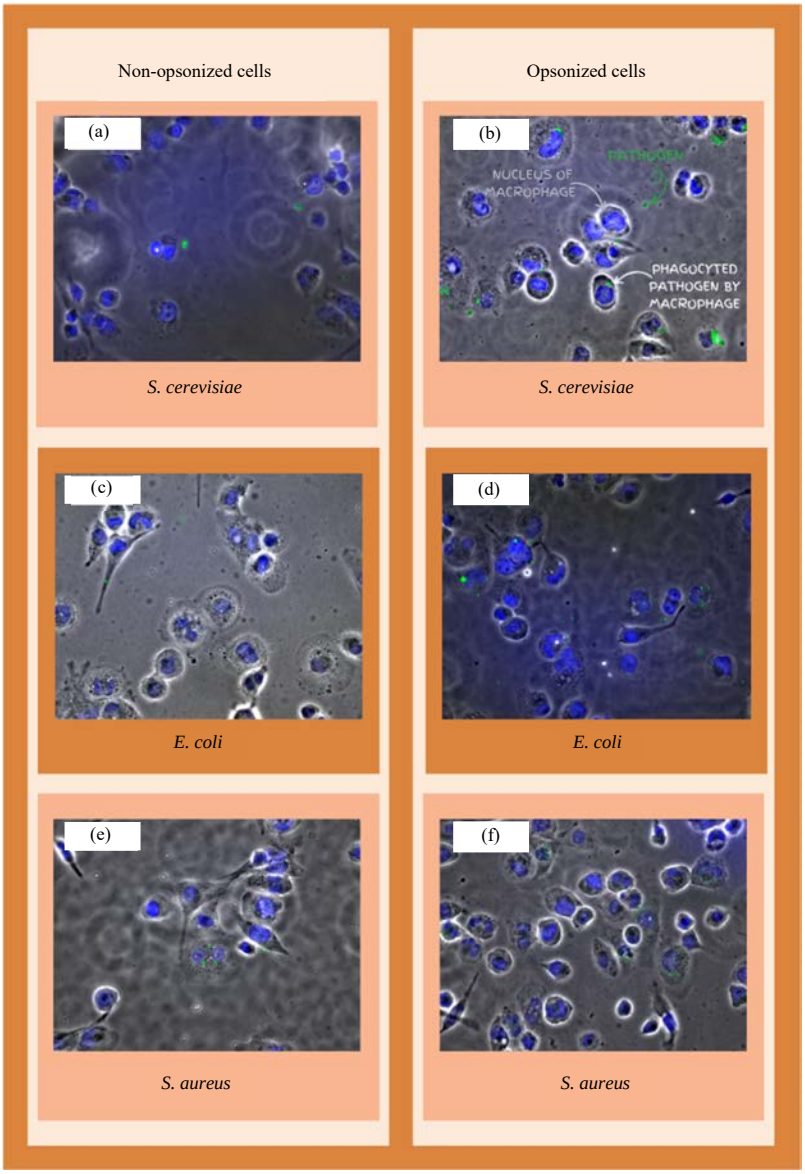

Fig. 1(a-f): Visual demonstration of phagocytosis under non-opsonised and opsonised conditions, analysed using ImageJ, (a) Non-opsonised *S. cerevisiae* cells showing limited uptake by macrophages, (b) Opsonised *S. cerevisiae* cells demonstrating increased uptake (green dots) within macrophages (blue nuclei), (c) Non-opsonised *E. coli* cells with low levels of phagocytosis, (d) Opsonised *E. coli* cells with markedly higher internalisation by macrophages, (e) Non-opsonised *S. aureus* cells showing moderate uptake and (f) Opsonised *S. aureus* cells with a substantial increase in phagocytic activity

Can be visible as green dots in the pictures. The host cell nucleus is shown as blue dots. Green phagocytes are visibly closer to the blue nucleus of host cells in opsonized specimens and they are visible and more abundant in numbers

Table 2: Statistical comparison of phagocytosis efficiency between opsonised and non-opsonised microorganisms

Organism	Parameter (t-value)	Statistical result
S. cerevisiae	t1	3.675249732
E. coli	t2	3.434536417
S. aureus	t3	4.746116731
p value	2.262	P<t (1,2,3)

DISCUSSION

The current study provides compelling evidence of the critical role opsonisation plays in enhancing phagocytic responses across a variety of microorganisms, including *Escherichia coli*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*. The data indicate that opsonisation, accomplished through the action of serum-derived complement proteins and antibodies, significantly boosts the phagocytic index of these pathogens. Opsonins act as molecular "handles", thereby increasing the efficiency with which phagocytes, particularly macrophages and neutrophils, can recognize and ingest pathogens[11]. In particular, complement C3b has been shown to trigger both phagocytosis and neutrophil activation via complement receptor 1 (CR1), illustrating the mechanistic basis for enhanced pathogen clearance following opsonisation[12]. This observation supports existing literature that emphasizes the indispensable nature of opsonins in the immune system's effort to clear pathogens.

The enhancement of phagocytosis observed in this study carries broader implications for immune response efficacy. Particularly noteworthy is the substantial increase in the phagocytic index for *S. cerevisiae*, a yeast, which highlights the immune system's capacity to effectively address more complex organisms. Given the structural complexity of yeast with its polysaccharide-rich cell wall, the results suggest a sophisticated interaction between opsonins and yeast's pathogen recognition receptors, such as Dectin-1, illustrating the diverse strategies employed by the immune system to cope with pathogen variability[13].

Furthermore, the study noted structural differences between gram-positive and gram-negative bacteria, which affected their immune recognition and response. This was especially evident in the case of *Staphylococcus aureus*, where the significant increase in the phagocytic index suggests that opsonisation successfully circumvents the robust peptidoglycan layer characteristic of gram-positive bacteria[14]. These findings deepen our understanding of how immune systems might be enhanced to overcome structural and compositional barriers in pathogens. Additionally, metabolic interactions between macrophages and internalized bacteria could further influence the success of phagocytosis, pointing to a multifactorial regulatory system[15-17].

However, it is important to acknowledge certain limitations of this study. The experimental design was restricted to a limited number of microorganisms and a single human serum concentration, which may not fully capture the complexity of immune interactions occurring *in vivo*. Moreover, while the microscopy-based quantification of phagocytosis provided valuable insights, it inherently introduces potential observer bias and variability due to manual image analysis. Future studies should consider employing flow cytometry or automated image-based analysis to achieve higher precision and reproducibility.

Additionally, future research could include a wider range of pathogens, explore different serum concentrations and investigate other immune cells such as neutrophils or dendritic cells. Integrating molecular techniques, such as receptor-blocking assays or transcriptomic profiling of phagocytes, could also uncover specific signalling pathways underlying opsonisation-enhanced phagocytosis. These improvements would deepen our understanding of how immune cells interact with pathogens and could guide the development of therapies that strengthen the body's natural defence mechanisms.

CONCLUSION

This study demonstrates that opsonisation markedly enhances phagocytic efficiency across all tested microorganisms, reinforcing its central role in innate immune defence. The findings suggest promising therapeutic avenues aimed at strengthening immune responses especially in immunocompromised individuals by leveraging opsonin-mediated enhancement of pathogen clearance. Insights gained here also hold relevance for vaccine strategies that rely on robust phagocytic activation. Continued research exploring opsonin–pathogen interactions and phagocyte genetic variability will further illuminate opportunities to optimise immune-targeted interventions.

SIGNIFICANCE STATEMENT

Phagocytosis is a fundamental component of the innate immune defence, primarily mediated by professional phagocytes such as macrophages and neutrophils. This study systematically evaluates the effect of human serum and opsonisation on the phagocytic uptake of three distinct microorganisms: *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive) and *Saccharomyces cerevisiae* (yeast). The findings highlight the critical role of opsonisation in enhancing microbial uptake, demonstrating how complement proteins and immunoglobulins in human serum act as molecular bridges that significantly amplify phagocytic efficiency.

REFERENCES

1. de Jong, N.W.M., K.P.M. van Kessel and J.A.G. van Strijp, 2019. Immune evasion by *Staphylococcus aureus*. Microbiol. Spectrum, Vol. 7. 10.1128/microbiolspec.gpp3-0061-2019.
2. Boero, E., R.D. Gorham Jr., E.A. Francis, J. Brand and L.H. Teng *et al*., 2023. Purified complement C3b triggers phagocytosis and activation of human neutrophils via complement receptor 1. Sci. Rep., Vol. 13. 10.1038/s41598-022-27279-4.
3. BoseDasgupta, S. and J. Pieters, 2018. Macrophage-microbe interaction: Lessons learned from the pathogen *Mycobacterium tuberculosis*. Semin. Immunopathol., 40: 577-591.
4. Rosales, C., and E. Uribe-Querol, 2017. Phagocytosis: A fundamental process in immunity. Biomed Res. Int., Vol. 2017. 10.1155/2017/9042851.
5. Sun, M., E. Arevalo, M. Strynar, A. Lindstrom and M. Richardson *et al*., 2016. Legacy and emerging perfluoroalkyl substances are important drinking water contaminants in the Cape Fear River Watershed of North Carolina. Environ. Sci. Technol. Lett., 3: 415-419.
6. Drevets, D.A., B.P. Canono and P.A. Campbell, 2015. Measurement of bacterial ingestion and killing by macrophages. Curr. Protocol. Immunol., Vol. 109. 10.1002/0471142735.im1406s109.
7. Flannagan, R.S., V. Jaumouillé and S. Grinstein, 2011. The cell biology of phagocytosis. Annu. Rev. Pathol.: Pathol. Mech. Dis., 7: 61-98.
8. Galli, G. and M. Saleh, 2021. Immunometabolism of macrophages in bacterial infections. Front. Cell. Infect. Microbiol., Vol. 10. 10.3389/fcimb.2020.607650.
9. Grinstein, S., C.J. Swallow and O.D. Rotstein, 1991. Regulation of cytoplasmic pH in phagocytic cell function and dysfunction. Clin. Biochem., 24: 241-247.
10. Gröndahl, G., A. Johannisson, M. Jensen-Waern and K.N. Ekdahl, 2001. Opsonization of yeast cells with equine iC3b, C3b, and IgG. Vet. Immunol. Immunopathol., 80: 209-223.
11. Koziel, J., A. Maciag-Gudowska, T. Mikolajczyk, M. Bzowska and D.E. Sturdevant *et al*., 2009. Phagocytosis of *Staphylococcus aureus* by macrophages exerts cytoprotective effects manifested by the upregulation of antiapoptotic factors. PLoS ONE, Vol. 4. 10.1371/journal.pone.0005210.
12. Meng, E.X., G.N. Verne and Q. Zhou, 2024. Macrophages and gut barrier function: Guardians of gastrointestinal health in post-inflammatory and post-infection responses. Int. J. Mol. Sci., Vol. 25. 10.3390/ijms25179422.
13. Murphy, K. and C. Weaver, 2016. Janeway's Immunobiology. 9th Edn., Garland Science, New York, ISBN: 9781315533247, Pages: 924.
14. Takeuchi, O., K. Hoshino, T. Kawai, H. Sanjo and H. Takada *et al*., 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity, 11: 443-451.
15. Westman, J. and S. Grinstein, 2021. Determinants of phagosomal pH during host-pathogen interactions. Front. Cell Dev. Biol., Vol. 8. 10.3389/fcell.2020.624958.
16. Xu, S., Y. Xiong, B. Fu, D. Guo, Z. Sha, X. Lin and H. Wu, 2023. Bacteria and macrophages in the tumor microenvironment. Front. Microbiol., Vol. 14. 10.3389/fmicb.2023.1115556.
17. Zhang, Z., Y. Wang, L. Xia and Y. Zhang, 2024. Roles of critical amino acids metabolism in the interactions between intracellular bacterial infection and macrophage function. Curr. Microbiol., Vol. 81. 10.1007/s00284-024-03801-x.