Molecular Characterisation of the Synovial Fluid Microbiome

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Abstract

Background: The term "microbiome" describes the genetic material encoding the various microbial populations that inhabit our body. In contrast, the term "microbiota" refers to the viable organisms that comprise these communities. Whilst the colonisation of specific body sites in contact with the external environment (such as the gastrointestinal tract, skin and vagina) by microorganisms is both well-described and universally accepted, the existence of microbial evidence in other "classically sterile" locations including the blood, synovial space, and lungs, is a relatively new concept. Increasingly, a role for the microbiome in disease initiation and progression is being considered, and it is therefore of upmost importance to increase our understanding of these complex communities. To date, no study has investigated the potential for a "synovial fluid microbiome" using the cutting edge molecular and bioinformatic techniques now available. To this end, we investigated the presence of bacterial and fungal DNA in the synovial fluid of rheumatoid arthritis (RA) patients and healthy control subjects. **Methods:** Synovial fluid samples from 25 subjects were analysed through amplification and sequencing of the 16S rRNA V4 and ITS2 genes followed by bioinformatic analysis. **Results:** Bacterial 16S rRNA was detected in fourteen out of sixteen (87.5%) RA patients, and nine out of nine (100%) healthy control subjects. Fungal DNA was identified in 12 out of 16 (75%) RA samples, and 8 out of 9 (88.8%) healthy controls. Bioinformatic analysis revealed evidence of a complex bacterial and fungal microbiome present in the synovial fluid of both healthy control subjects and RA patients. **Concluding remarks:** Through taxonomic classification of sequencing reads we describe a complex community of bacterial and fungal DNA present in the synovial fluid of both healthy control subjects and RA patients. Through statistical analysis of read abundances, we identify key taxa that are differentially present or abundant dependent on disease status. This is the first study to report the synovial fluid microbiome, and to determine that this is modulated by disease status (RA) as are other classical microbiome niches.

Introduction

The Microbiome

The term “microbiome” describes the genetic material encoding the various microbial populations that inhabit our body. In contrast, the term “microbiota” refers to the viable organisms that comprise these communities. Cell for cell, bacterial cells outnumber our own cells by a factor of ten, and the bacterial microbiome encodes for one hundred-fold more genes. The microbiome undertakes essential biological processes and thus it is unsurprising that a number of disease states are associated with changes in microbiome composition, termed “dysbiosis”. Whilst the colonisation of specific body sites in contact with the external environment (such as the gastrointestinal tract, skin and vagina) by microorganisms is both well-described and universally accepted [1], the existence of microbial DNA in other “classically sterile” locations including the blood [2], synovial space, and lungs, is a relatively new concept.

Rheumatoid Arthritis and Dysbiosis

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes synovial joint inflammation leading to significant joint pain, swelling, and significant disability with increased morbidity and mortality. RA is estimated to affect ~1% of the population worldwide [3]. Increasingly, a role for the microbiome in disease initiation and progression is being considered. To date, RA has been associated with dysbiosis of the oral [4-7], gastrointestinal [6-14], and respiratory [15-17] microbiomes and associated with the presence of specific organisms (most commonly *P. mirabilis*) within the urinary tract [18-21], suggesting that RA results in (or from) pan-microbiome dysbiosis. Studies have further begun to associate RA, or stages thereof, with the presence or absence of specific bacteria, suggesting that the microbiome may afford a valuable source of novel biomarkers and or novel targets for therapeutic modulation.

Evidence of Bacteria in the Synovial Fluid: Origin and Transport

Under normal physiological conditions, one expects the synovial space to be sterile. Nevertheless, numerous studies report the presence of microorganisms, or microorganism-derived genetic material in the synovial fluid of RA patients and healthy control subjects [22-28]. Interestingly, these studies overwhelmingly identify bacteria most commonly found to reside within the oral cavity, and these findings are accompanied by evidence demonstrating the presence of these DNAs and or antibodies directed against the originating organism in the blood [22, 23, 29-32]. On the basis of these findings, we suggest that bacterial DNAs may reach the synovial space via the blood (as evidenced by their concurrent presence in both fluids), and further, that the inflammatory environment of the synovial tissues may facilitate the trapping of these bacterial DNAs, increasing their apparent concentration in this location [23].

Despite extensive evidence linking infection of the oral tissues with RA [4, 5, 15, 33-42], and data demonstrating the translocation of microbial DNA from distant microbiome niches to the synovial space (see above), there has been no global characterisation of the bacterial and fungal DNA present this location to date. To this end, we investigated the presence of bacterial (via sequencing of the 16S rRNA gene) and fungal (via sequencing of the ITS2 region) DNA, in the synovial fluid of human RA patients and healthy control subjects using cutting-edge next generating sequencing and bioinformatic techniques.

Materials and Methods

Donor Population

This study investigated the presence of bacterial and fungal DNA in synovial fluid samples from fifteen rheumatoid arthritis patients, and nine sex and BMI-matched disease-free individuals. Synovial fluid samples were aspirated from the knee joint using aseptic technique, transmitted to a sterile micro-centrifuge tube, and stored at -80°C prior to further analysis. Samples were procured from Sera Laboratories Limited. All samples were analysed anonymously, and the authors obtained ethical approval (Keele University ERP3) and written informed consent to utilise the samples for the research reported herein.

Microbiome Characterisation

Amplification and sequencing of the bacterial 16S rRNA variable region 4 and fungal ITS region was used to characterise the bacterial and fungal DNA present in the synovial fluid samples.

The bacterial 16S rRNA and fungal ITS2 genes were amplified by PCR utilising the oligonucleotide primers detailed in (**Table 1)**. A first round of PCR using oligonucleotide primers 16SV4 F/R and ITS2 F/R was carried out with four microliters of each synovial fluid sample as template in a final volume of 20µl. Reactions comprised 10µl Phusion blood PCR buffer (Thermofisher), 0.4µl (2 U) Phusion blood DNA polymerase, 1µl of each primer (10µM), and 3.6µl of nuclease-free water that had been subject to 15 minutes UV-irradiation. A negative control reaction in which synovial fluid was substituted with UV-irradiated nuclease-free water was included in each experiment to confirm that none of all reagents were contaminated by target DNA.

Cycling parameters comprised an initial denaturation step performed at 98°C for 5 minutes, followed by 33 cycles of denaturation (98°C, 10 seconds), annealing (55°C, 5 seconds) and extension (72°C, 15 seconds). A final elongation of 7 minutes at 72°C completed the reaction. Following gel electrophoresis of 5µl of each resulting PCR product to confirm successful amplification, the remainder (15µl) was purified of excess primer and PCR reagents using the QIAquick PCR Purification kit. A “kit control” was run alongside this process and involved the purification of 15µl of UV-irradiated nuclease-free water, again to ensure the kit used was free from contaminating DNA.

In order to add sequencing adapters to facilitate MiSeq library preparation, a second round of PCR amplification was conducted in a total volume of 50µl comprising 10µl 5X Platinum Super-Fi Buffer, 1µl 10mM dNTP mixture, 0.5µl Platinum Super-Fi polymerase, 2.5µl of each XT_tagged primer (16SV4_XT F/R or ITS2_XT F/R), 5µL from the successful first round PCR reaction and 38.5 µL of UV-irradiated nuclease-free water. Cycling parameters comprised an initial denaturation step performed at 98°C for 2 minutes, followed by 7 cycles of denaturation (98°C, 10 seconds), and

annealing / extension (72°C, 20 seconds) extension (72°C, 15 seconds). A final elongation of 5 minutes at 72°C completed the reaction. PCR products were purified using AMPure XP magnetic beads (Agencourt) at a ratio of 0.8 beads to sample (v/v), eluted in 20µl of UV-irradiated nuclease-free water, and quantified using the Qubit 3.0 high-sensitivity DNA kit (Invitrogen).

Amplicons were barcoded using the Nextera DNA library kit, multiplexed for efficiency, and sequenced using the Ilumina MiSeq system with a 250bp paired-end read metric. Bioinformatic analysis was performed using QIIME implemented as part of the Nephele 16S / ITS paired-end QIIME pipeline using open reference clustering against the SILVA database for bacteria and the ITS database for fungi at a sequence identity of 99%. All other parameters remained as default. The statistical significance of differences in the abundance of individual bacteria and fungi between the RA and control donors was determined by unpaired t test, following confirmation that our data approximated the “normal distribution”.

Table1: Oligonucleotide primers used in this study

Primer Name	Primer Sequence (5’ – 3’)	Length
16SV4_F	GTGCCAGCMGCCGCGGTAA	19
16SV4_R	GGACTACHVGGGTWTCTAAT	20
16SV4_XT_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGC CGCGGTAA	52
16SV4_XT_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVG GGTWTCTAAT	54
ITS2_F	GCA TCG ATG AAG AAC GCAGC	20
ITS2_R	TCCTCCGCTTATTGATATGC	20
ITS2_XT_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCATCGATGAAGAACGCAGC	53
ITS2_XT_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCTCCGCTTATTGATATGC	54

Results and Discussion

Clinical features of donors and results of 16S rRNA and ITS2 PCR amplification

Synovial fluid samples were obtained from 25 human donors. Sixteen patients were diagnosed with RA, and of these, 8 were male and 8 were female. The RA patients' ages ranged from 52 to 74 years with a mean of 65.3 years. Nine control synovial fluid samples were obtained from 5 males and 4 females. Their ages ranged from 50 to 68 years with a mean of 61.0 years. There were no significant differences in the ages of the two patient cohorts (P = > 0.05) **(Table 2**).

Utilising PCR amplification, bacterial 16S DNA was detected in the synovial fluid of 14 of out 16 patients with RA (87.5%), and in 9 out of 9 (100%) healthy control subjects. ITS2 amplification, indicative of the presence of fungi, was detected in 12 of 16 (75%) RA samples, and 8 out of 9 (89%) healthy control samples. Our various experimental controls (template free / kit controls) consistently failed to produce a visible band following PCR and agarose gel electrophoresis. Furthermore, DNA quantification using the Qubit 3.0 high-sensitivity DNA kit (Invitrogen), confirmed this absence.

Table 2: Characteristics of the patient population.

Patient ID#	Gender	Age	Diagnosis	16S rRNA PCR	ITS2 PCR
001	Female	65	RA	+	−
002	Female	67	RA	+	+
003	Female	67	RA	+	+
004	Female	67	RA	+	−
005	Female	70	RA	+	−
006	Female	69	RA	+	−
007	Male	56	RA	+	+
008	Male	52	RA	+	+
009	Male	55	RA	+	+
010	Male	66	RA	+	+
011	Male	74	RA	+	+
012	Male	69	RA	+	+
013	Female	67	RA	+	+
014	Female	67	RA	−	+
015	Male	67	RA	−	+
016	Male	67	RA	+	+
017	Female	57	Healthy	+	+
018	Male	64	Healthy	+	−
019	Male	64	Healthy	+	+
020	Male	53	Healthy	+	+
021	Male	50	Healthy	+	+
022	Male	74	Healthy	+	+
023	Female	64	Healthy	+	+
024	Female	55	Healthy	+	+
025	Female	68	Healthy	+	+

Characterisation of Bacterial populations via 16S rDNA sequencing of synovial fluid

The presence of bacterial DNA in the synovial fluid was evaluated via PCR amplification and sequencing of the bacterial 16S rRNA gene, variable region 4. An average of 75,000 reads were generated for each of the samples; 70,658 reads in the RA samples, and 80,533 reads in the Control samples. Although the control samples generated more reads on average, this difference was not statistically significant (P = > 0.05), and nevertheless, rarefaction was used prior to differential expression analysis. Following taxonomic classification using the Nephele platform as described above, our first approach utilised Principal Coordinates Analysis (PCoA) to reduce the complexity of the data obtained, and to visualise any obvious separation between the two experimental groups **(Figure 1).**

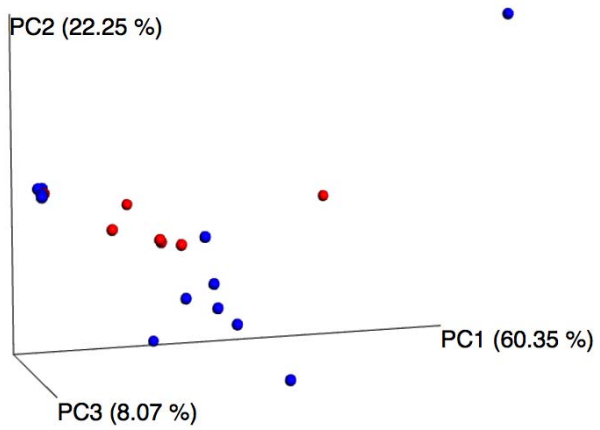

Figure 1 - Principal Coordinate Analysis plot generated from a Bray Curtis distance matrix of synovial fluid bacterial community structure for control subjects (red) and arthritic patients (blue) as determined via amplification and sequencing of the 16S rRNA variable region 4 (V4). Proportions of variation explained by the principal coordinates are designated on the axes. PCoA found that the maximal variation were 60.31% (PC1), 22.23% (PC2) and, 8.12% (PC3). The microbiome of samples that appear in close proximity to each other are more similar in composition.

At the phylum level, the synovial fluid microbiome was predominated by Proteobacteria (81%) and Firmicutes (16.5%), and to a much lesser extent, Actinobacteria (0.3%) and Bacteroidetes (0.1%). Whilst the major phyla identified appear similar to those of the blood microbiome [2], they are present in the synovial fluid at different levels suggesting a distinct community structure.

At the genus level, our synovial fluid samples were predominated by the genus *Pseudomonas* (RA = 66.1%, Control = 80.6%), followed by the genus *Enterococcus* (RA = 11.4%, Control = 11.7%). To a lesser extent, synovial fluid samples contained taxa of the *Bacillales* order (RA = 7.4%, Control = 4%), and *Stenotrophomonas* genus (RA = 4.2%, Control = 1.5%). The genera *Raoultella* (RA = 6.5%,) and *Bacillus* (RA = 1.2%,) were detected exclusively in the synovial fluid of our RA patients **(Figure 2 and 3)**. This community structure is distinct from our previous report on the blood microbiome [2].

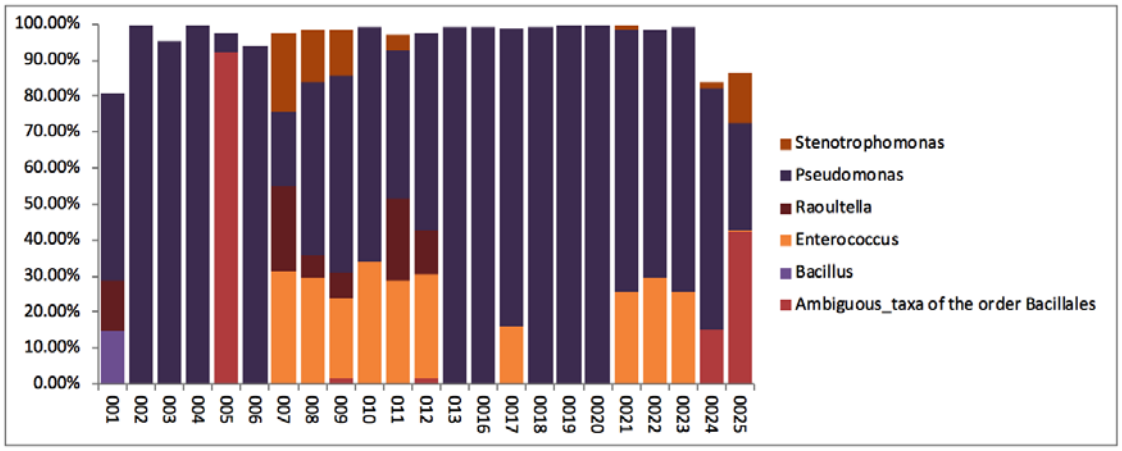

Figure 2 - Relative abundance of genera representing >1% of the total bacterial population in synovial fluid samples of rheumatoid arthritis (RA, n = 14) and control (Control, n = 9) samples as determined by amplification and sequencing of the 16S rRNA gene variable region 4. Data are mean abundance expressed as a percentage of the total bacterial sequence count.

Statistical analysis revealed that whilst the abundance of most bacterial DNA was unaffected by disease state, the abundance of *Raoultella* was borderline significant; observed as the selective presence of this organism in the synovial fluid of RA patients only (**Table 3 and Figure 3**).

With regards the numerous reports detailing a link between oral and urinary tract associated bacteria and RA, we reviewed the results of our taxonomic classification looking specifically for the presence and abundance of these organisms. Bacteria of the genus *Porphyromonas* were largely absent with the exception of three synovial fluid samples (RA =1, Control = 2) which returned a small number of reads mapping to this genus. No reads mapping to the genus *Proteus* were identified in any sample analysed, however numerous other members of the order Enterobacteriales to which *Proteus* belongs were identified, including *Raoultella*. It is important to note here that none of our RA patients reported oral or urinary conditions, and it is therefore plausible that the above bacteria are present only during active oral or urinary disease. A separate study is perhaps warranted to further investigate this phenomena including RA patients (without co-morbidities), RA patients with poor oral health and patients with periodontitis alone (without RA).

Table 3 Statistical analysis of the relative abundance of common bacterial genera, with expression levels >1% of the total bacterial read population, in Control and RA synovial fluid samples. Data are mean percentage ± standard error of the mean (SEM). P values were determined by unpaired t-test following confirmation of data normality. P ≤ 0.05 was considered statistically significant.

Taxa	Control abundance (%)	RA abundance (%)	P value
Order			
Bacillales	6.37 ± 6.13	6.4 ± 6.48	0.97
Genus			
Stenotrophomonas	3.8 ± 1.9	1.9 ± 1.5	0.97
Pseudomonas	66.4 ± 8.5	77.1 ± 7.4	0.30
Raoultella	0.08 ± 0.03	6.11 ± 2.31	**0.051**
Enterococcus	10.76 ± 4.39	12.54 ± 4	0.95
Bacillus	0 ± 0	1.04 ± 1.04	0.99

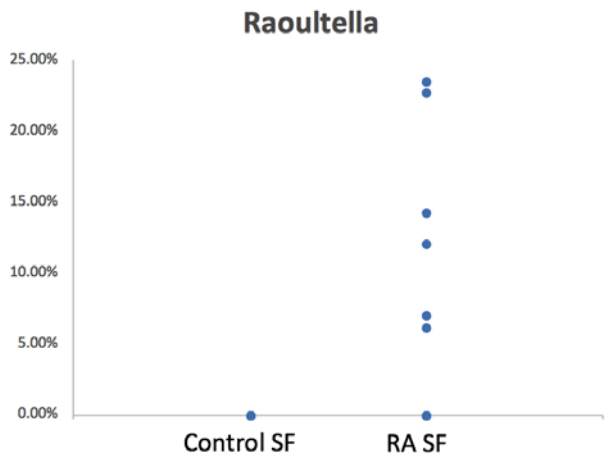

Figure 3 - Relative abundance of the genus *Raoultella*, identified in the synovial fluid of healthy donors and rheumatoid arthritis patients by amplification and sequencing of the 16S rRNA gene variable region. A borderline significant ($p = 0.051$) increase in the mean abundance of *Raoultella* was identified in RA synovial fluid relative to healthy control synovial fluid. In fact, *Raoultella* was only (selectively) present in the synovial fluid of our RA patients, and absent from healthy control synovial fluid. Data are individual sample abundances expressed as a percentage of the total bacterial sequence count. Statistical significance was determined by unpaired t test with $P < 0.05$ considered significant.

Characterisation of fungal populations via ITS2 sequencing of synovial fluid

The presence of fungal DNA in synovial fluid was evaluated via PCR amplification and sequencing of the fungal ITS2 gene. An average of 50,000 reads were generated for each of the samples; 52,113 reads in the RA samples, and 49,929 reads in the Control samples. Although the RA samples generated more reads on average, this difference was not statistically significant (P = > 0.05). Following taxonomic classification using the Nephele platform as described above, our first approach utilised Principal Coordinates Analysis (PCoA) to reduce the complexity of the data obtained, and to visualise any obvious separation between the two experimental groups **(Figure 4).**

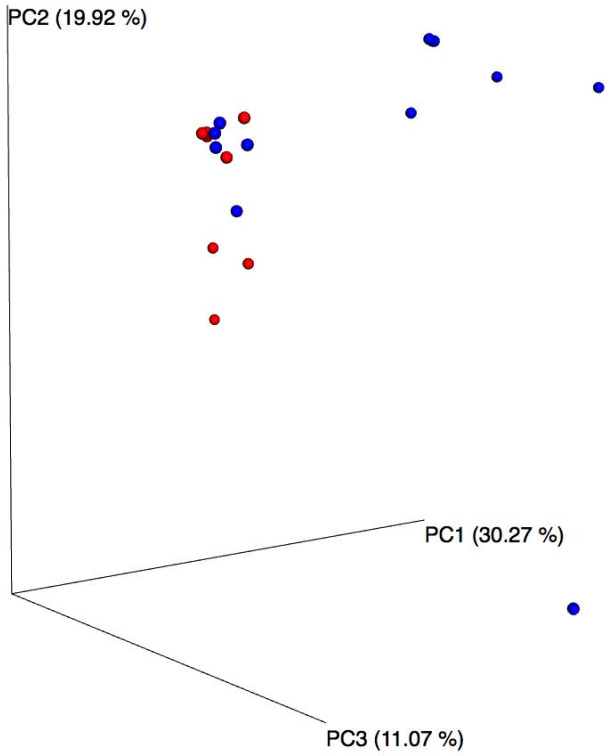

Figure 4 - Principal Coordinate Analysis plot generated from a Bray Curtis distance matrix of synovial fluid bacterial community structure for control subjects (red) and arthritic patients (blue) as determined by amplification and sequencing of the ITS2 gene. Proportions of variation explained by the principal coordinates are designated on the axes. PCA found that the maximal variation were 30.27% (PC1), 19.92% (PC2) and, 11.07% (PC3). The microbiome of samples that appear in close proximity to each other are more similar in composition.

At the phylum level, synovial fluid was found to be predominated by members of the Basidiomycota (50.4%) and Ascomycota (35.1%) phyla. At the genus level, synovial fluid samples were predominated by the genus *Malassezia*, which accounted for 40.9% and 45.3% of the total number of fungal sequences identified in the RA and Control donors respectively. To a lesser extent, synovial fluid samples also contained the classes *Tremellomycete*s (RA = 2.8%, Control = 3%), *Agaricomycetes* (2.2%, 3.2%), and *Penicillium* (0.7%, 15.2%). Members of the *Hypocreales* order (RA = 26.4%,), and the genus *Aspergillus* (RA = 6.2%,), were detected in RA synovial fluid samples only. Further, the order *Malasseziale*s (1.2%), and genera *Candida* (, Control = 11.9%), *Pichia* (, Control = 8.9%), *Cladosporium* (, Control = 5.1%), and *Debaryomyces* (, Control = 4.7%) were observed in control synovial fluid only.

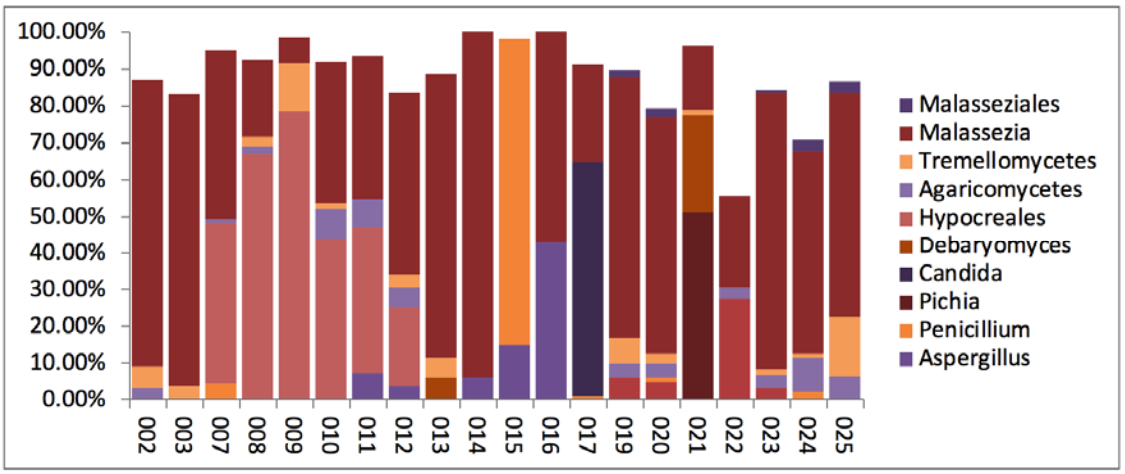

Figure 5 - Relative abundance of taxa representing >1% of the total fungal population in each synovial fluid sample (RA, n = 12) and (Control, n = 9) as determined by amplification and sequencing of the fungal ITS2 gene. Data are mean abundance expressed as a percentage of the total fungal sequence count.

Statistical analysis revealed that whilst the abundance of most fungal DNA was unaffected by disease state, select organisms were differentially abundant or present (**Table 4** and **Figure 6**). Members of the *Hypocreales* order and the genus *Aspergillus* were detected in RA synovial fluid samples only (P = 0.012 and 0.057 respectively). Further, the order *Malasseziale*s (P = 0.002), and genus *Cladosporium* (P = 0.019) were observed in control synovial fluid only.

Table 4 - Statistical analysis of the relative abundance of common fungal genera with expression levels >1% of the total fungal sequence count, in control and RA synovial fluid samples. Data are mean percentage ± standard error of the mean (SEM). P values were determined by unpaired t-test following confirmation of data normality. P ≤ 0.05 was considered statistically significant.

Taxa	Control abundance (%)	RA abundance (%)	P value
Class			
Tremellomycetes	3.81 ± 1.91	3.21 ± 1.18	0.98
Agaricomycetes	3.71 ± 1.07	2.23 ± 0.97	0.3
Order			
Hypocreales	0±0	24.59 ± 8.38	**0.012**
Malasseziales	1.48 ± 0.47	0.12 ± 0.12	**0.002**
Genus			
Aspergillus	0±0	6.79±3.88	**0.057**
Cladosporium	0.08 ± 0.05	5.27 ± 3.28	**0.019**
Malassezia	49.4 ± 8	46.1± 8.9	0.8
Penicillium	0.57 ± 0.3	7.98 ± 7.53	0.6
Pichia	6.425 ± 6.42	0±0	0.4
Debaryomyces	3.26 ± 3.26	0.5± 0.5	0.7
Candida	7.9 ± 7.9	0±0	0.4

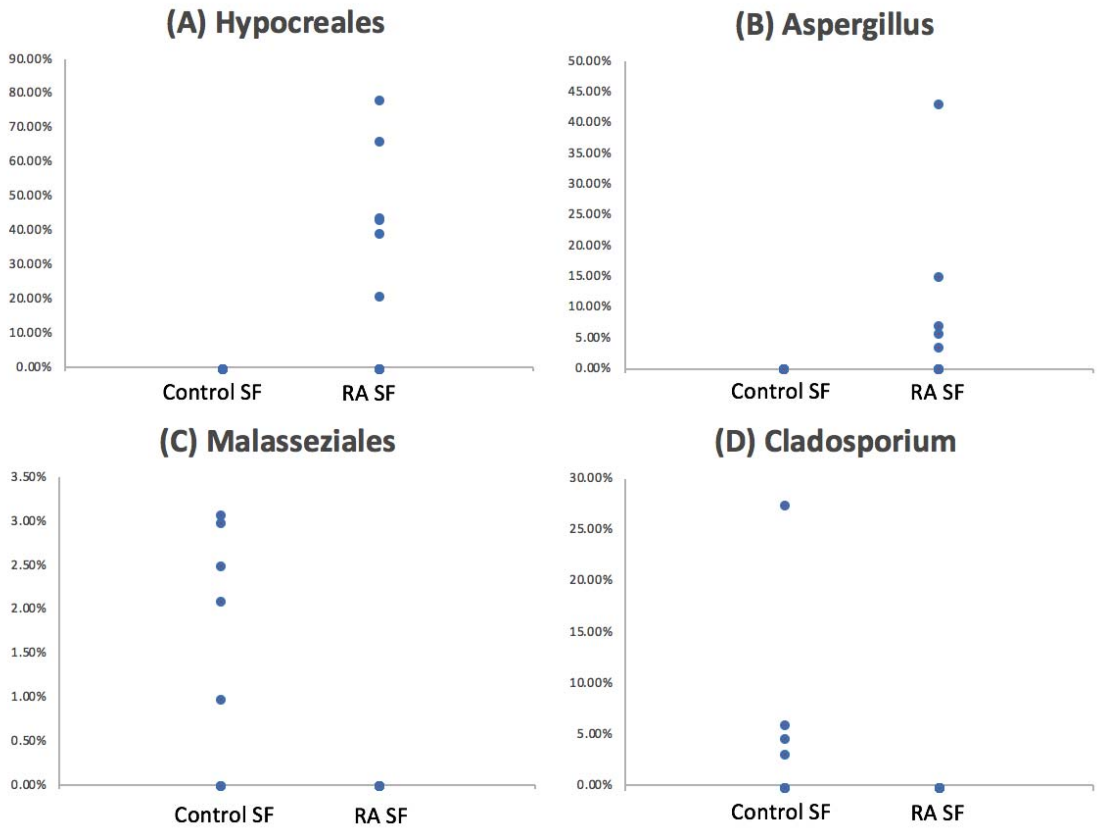

Figure 6 - Relative abundance of (A) *Hypocreales*, (B), *Aspergillus*, (C) *Malasseziales*, and (D) Cladosporium identified in the synovial fluid of rheumatoid arthritis patients and healthy control subjects. Data determined by the amplification and sequencing of the ITS2 gene. (A) The relative abundance of order *Hypocreales* was increased in synovial fluid of RA patients relative to healthy control synovial fluid. (B) The relative abundance of genus Aspergillus was increased in RA synovial fluid samples compared with healthy subjects (borderline significant). (C), and (D) The abundance of the order *Malasseziales* and genus *Cladosporium* were significantly decreased in the synovial fluid of RA patients in comparison to the synovial fluid of healthy control subjects. Data are individual expression values expressed as a percentage of the total fungal read count in each sample. The statistical significance between groups was determined by unpaired t test where $P \leq 0.05$.

Discussion

Mounting evidence suggests pan-microbiome dysbiosis in patients with rheumatoid arthritis. Moreover, various studies spanning many decades link the development of RA with infections of the oral tissues or urinary system, and report the presence of microorganisms or microorganism-derived genetic material in the synovial fluid; a compartment classically considered to be sterile. To our surprise, no study has investigated the potential for a “synovial fluid microbiome” using the cutting edge molecular techniques now available for our disposal. To this end, we investigated the presence of bacterial and fungal DNA in the synovial fluid of rheumatoid arthritis (RA) patients and healthy control subjects through amplification and sequencing of the 16S rRNA V4 and ITS2 genes.

Synovial fluid samples from 25 subjects were analysed in this study and our analyses revealed the presence of a complex synovial microbiome community in health and disease. Bacterial 16S rRNA was detected in fourteen out of sixteen (87.5%) RA patients, and nine out of nine (100%) healthy control subjects. Whilst the bacterial communities characterised in the disease and control states were largely unchanged, the presence of the genus *Raoultella* was restricted to the RA synovial fluid in our study (6 / 13 subjects, P = 0.051). Interestingly, when present, *Raoultella* occupied a large proportion of the total bacterial read count (14.27%). This is the first study to describe the genus *Raoultella* in RA synovial fluid samples, however, two major species are capable of causing infections in man: *Raoultella ornithinolytica* and *Raoultella planticola*, and have been shown to be capable of reaching the articular joint compartment [43-45]. It is plausible that the presence and or indeed abundance of *Raoultella* may correlate with specific disease features (for example; disease stage, severity, current disease activity, treatment regimen), and we therefore plan to explore this relationship further in the future.

Fungal DNA was identified in 12 out of 16 (75%) RA samples, and 8 out of 9 (88.8%) healthy controls. Again we identified key taxa that appeared to be differentially present or abundant dependent on disease status. The order Hypocreales was selectively abundant in RA patients (6 / 12) and absent from healthy control synovial fluid. Interestingly, when present, members of the Hypocreales order occupied a large proportion of the total fungal read count (49.1%). Evidence of members of this order have been previously identified in the healthy human oral cavity [46] and found to be ubiquitous in human plasma [47], however no reports directly link this order with RA.

Further, the abundance of genus *Aspergillus* was borderline significant; detected as a selective presence in the synovial fluid of RA patients only (5 / 12) and occupying an average of 14.9% of the read population when present. *Aspergillus* has been identified in the healthy human gut [48] and oral cavity [46] in various studies, and is a prevalent opportunistic microorganism of the lungs, particularly affecting immunocompromised individuals, where it causes a range of pulmonary diseases [49]. Interestingly, treatment with Adalimumab, an anti-TNF drug used to treat various inflammatory conditions (including rheumatoid arthritis), has been associated with various fungal infections, including Aspergillosis [50-54]. The relative abundances of the order Malasseziales and genus Cladosporium were absent in the synovial fluid of all RA patients in comparison to the synovial fluid of healthy control subjects (present in 4 / 8 and 5 / 8 healthy control patients respectively). Members of the Malasseziales order have been identified in the healthy human gut [48] and further, isolated from the blood of disease-free individuals [55]. Moreover, this order was found to be decreased in the case of inflammatory bowel disease [56], suggesting it may be responsive to an inflammatory environment. Cladosporium has been shown to colonise the healthy human gut [48] and oral cavity [46], and is encountered commonly in human clinical samples [57], thus evidence of Cladosporium DNA in the healthy patient is perhaps not unexpected. That said, it's absence from the synovial fluid of all RA patients may suggest an altered fungal community in some distant microbial niche, that presents as a reduction in translocated Cladosporium DNA. These findings all warrant further investigation.

Concluding Remarks

This is the first study to report the synovial fluid microbiome, and to determine that this is modulated by disease status (RA) as are other classical microbiome niches. Our recent work on the blood microbiome [2], and that of others [58-68], characterised a complex community of organisms (or the genetic material thereof, more specifically) that likely originate in one of the classical microbiome niches (the gut, mouth, urogenital tract, skin) and reach the circulation through a process termed atopobiosis. We suggest that the same process may be true of the community we characterise here in the synovial fluid, with the circulatory system acting as a conduit between their usual place of habitation and the synovial compartment.

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