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Potential of Clinical Care-Collected Gut Biopsies for Advancing Personalised Medicine in Inflammatory Bowel Disease

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ABSTRACT

The management of inflammatory bowel disease (IBD) is challenged by significant patient diversity and a lack of effective profiling tools. This review evaluates the potential of archived formalin-fixed, paraffin-embedded (FFPE) gut biopsies as a resource for translational and precision medicine, given their global availability and associated long-term clinical data. Recent technical advances have expanded the molecular toolbox for FFPE samples to include proteomics, metabolomics, glycomics, spatial transcriptomics and characterising microbial components via 16S rRNA sequencing. Despite their potential, the use of FFPE materials is hindered using heterogeneous collection and storage methods. Consequently, the authors recommend implementing standardised guideline protocols for routine IBD biopsy management to facilitate high-quality research, improve disease understanding and support the identification of clinically translatable biomarkers.

1 | Introduction

The management of inflammatory bowel disease (IBD) remains challenging due to its heterogeneity. Molecular phenotyping offers a promising approach to defining individual disease profiles and enabling personalised management strategies by developing biomarkers with diagnostic, prognostic, predictive, and monitoring potential [1].

Formalin-fixed, paraffin-embedded (FFPE) tissue specimens from diagnostic and surgical procedures are widely archived in pathology laboratories and hospitals worldwide. They represent large real-world patient cohorts with long-term clinical follow-up data. Research strategies that use real-world biological samples may serve as a valuable complement to interventional clinical trials, which are time-consuming, costly, labour-intensive, and selective in patient recruitment. Real-world

Abbreviations: AI, artificial intelligence; DESI-MSI, Desorption Electrospray Ionization MSI; ECCO, The European Crohn's and Colitis Organisation; FFPE, Formalin-fixed, paraffin-embedded (FFPE); H&E, hematoxylin and eosin; IBD, inflammatory bowel disease; LCM, Laser Capture Microdissection; LMD, Laser microdissection; MALDI-MSI, matrix-assisted laser desorption ionization MSI; MSI, mass spectrometry imaging; PCR, Polymerase Chain Reaction; PNGase, Peptide:N-glycosidase; polyA, polyadenylated; qPCR, quantitative/real-time PCR; SNOMED, Systematised Nomenclature of Medicine.

data may include patient subpopulations that are underrepresented in conventional trials, potentially improving the generalizability and applicability of study outcomes to real-world conditions. Therefore, we are exploring herein the potential of using stored gut biopsies in IBD research (Figure 1).

We provide an in-depth examination of the potential of FFPE biopsies as a resource for translational and precision medicine. Particular emphasis is placed on molecular analyses that can be performed on archived samples, including the characterisation of microbial components within tissue. Our discussion then considers the broader opportunities offered by stored FFPE material for advancing personalised healthcare, alongside the methodological and ethical considerations that must be addressed when using such samples in research. Together, these sections underscore the central role of FFPE tissue repositories in shaping future omics-driven strategies for individualised medicine.

2 | Molecular Analyses Using FFPE Biopsies

Recent technical advances have expanded the molecular toolbox for FFPE samples beyond traditional histology and immunohistochemistry, incorporating metabolomics, proteomics, and glycomics. However, FFPE samples also pose unique technical challenges that can affect data quality and interpretability.

Proteomic analysis of FFPE tissues has become increasingly feasible due to the development of methods for efficient protein retrieval and digestion from cross-linked material (e.g., antigen retrieval). Technologies such as laser capture microdissection and mass spectrometry imaging (MSI) enable spatially resolved proteomic profiling, preserving tissue architecture and providing cell-type-specific insights [2]. Targeted and untargeted mass spectrometry workflows for protein detection have been optimised for FFPE, although protein cross-linking and degradation remain significant challenges. Formalin-induced modifications can affect peptide identification and quantification, and the degree of protein retrieval can vary across tissue regions. Despite these limitations, spatial proteomics has been successfully applied to gut biopsies to investigate inflammatory diseases [3].

Until recently, the extraction of metabolite information from FFPE specimens remained underexplored, largely due to concerns that fixation and embedding processes could chemically alter or degrade metabolites, leading to metabolite loss or misrepresentation. However, initial evidence suggests that specific metabolites can remain chemically and spatially conserved in FFPE tissues, making them suitable for molecular analysis. Histology-based MSI has been demonstrated to be a viable approach for label-free, multiplexed detection and spatial mapping of metabolites directly within their histological context, offering a promising tool for retrospective molecular investigations of archived tissue samples [4]. Especially, spatial metabolomics with MSI using FFPE tissue is an upcoming but technically constrained field. While fresh-frozen tissue remains the standard for metabolomics, recent adaptations of MALDI-MSI (matrix-assisted laser desorption ionization MSI) and DESI-MSI (Desorption Electrospray Ionization MSI) have shown that a subset of metabolites, particularly lipids and small apolar metabolites, can be detected in FFPE sections [5]. The main challenges arise from degradation, chemical modification during fixation and washout of metabolites during embedding [4]. However, the preserved spatial context can be particularly valuable for exploring metabolite distribution along crypt-villus

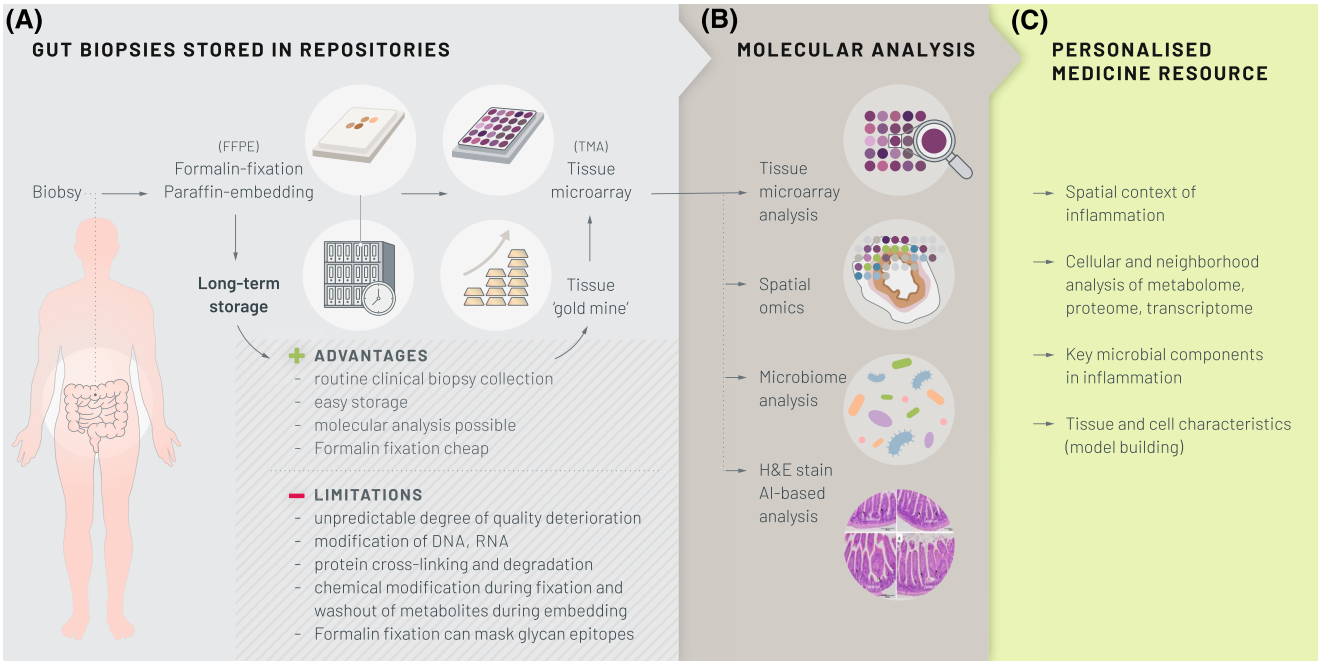

FIGURE 1 | Using FFPE tissue stored in repositories to advance personalised medicine. This figure illustrates the process from (A) routine collection and storage of gut biopsies in repositories (FFPE) to (B) their application in advanced molecular analyses (e.g., metabolomics, proteomics, spatial transcriptomics) for (C) developing personalised medicine resources in IBD, such as biomarkers and delineation of disease trajectories.

axes or in proximity to inflammatory lesions. Coupled with multiplexed imaging, spatial metabolomics offers insights into metabolic reprogramming, drug fate, and host-microbe interactions within the gut, although it currently offers lower sensitivity and depth than fresh-frozen tissue [6, 7].

A crucial molecular group central to cellular interactions is the glycans of cell membrane proteins. Analysis of glycans from FFPE tissue is less developed but gaining interest, especially in the context of gut biology, where glycans play key roles in host-microbe interactions and mucosal immunity [8]. Glycans can be targeted by MSI approaches from FFPE tissues, including on-tissue enzymatic reactions (e.g., PNGase (Peptide:N-glycosidase)) to make glycans amendable for MSI analysis [9]. Formalin fixation can mask glycan epitopes or alter glycosylation patterns, complicating interpretation. However, emerging workflows provide insights into spatial glycan mapping and may complement proteomic and metabolomic data.

FFPE biopsies present a unique opportunity for spatially resolved molecular analyses that integrate the histopathological context with biochemical insights. Using FFPE tissue for spatial transcriptomics to gain fundamental biological insights is a powerful method for understanding molecular processes in great detail, as newer approaches enable micrometre-scale spatial profiling. This method is on its way to allow higher throughput and therefore enables cohort-wide screening. The drawback of degraded RNA, including the loss of polyadenylated (polyA) tails that are critical for mRNA stability and sequencing, is well recognised. To mitigate these limitations, protocols originally developed for fresh-frozen tissue and other low-quality samples have been adapted for use [10, 11].

Together, the new molecular and omics-focused approaches are transforming FFPE tissue from a static archive into a dynamic source of molecular information, with increasing potential for clinical research and mechanistic discovery.

3 | Characterisation of Microbial Components in FFPE Tissue

Bacterial components in FFPE biopsies can be partially characterised and are particularly relevant in IBD, where microbial readouts provide critical insights into host-microbial interactions, dysbiosis, and their contribution to disease pathogenesis. FFPE tissues offer valuable genetic material for retrospective microbiome studies using 16S rRNA sequencing. An overview of published microbiome studies that utilised FFPE tissues, including for example, colorectal cancer specimens and neonatal intestinal samples, was recently provided by Cruz-Flores et al. [12] However, the application of FFPE samples to microbiome sequencing presents significant technical challenges that must be carefully considered and limit the overall feasibility.

FFPE-induced alterations are particularly detrimental to microbial analyses, as reduced DNA sequence quality and increased chimeric sequences can lead to misassigned taxa, thereby fundamentally compromising microbiome analyses. Specifically, the FFPE procedure can alter the structure and solubility of the mucins that comprise mucus, potentially affecting the mucosal microbial composition. Additionally, FFPE tissues typically contain low microbial content, increasing susceptibility to background/environmental noise and reagent contamination. At the same time, co-extraction of highly abundant host DNA further complicates the detection of microbial signals [13]. With respect to this, several studies have shown that environmental contaminants from laboratory reagents, paraffin, and extraction kits can distort results [12, 13]. Thus, the inclusion of different negative and positive controls, as well as the usage of (computational) decontamination protocols, is highly recommended and crucial for the interpretation of results. Though to the best of our knowledge, there is currently no particular in silico decontamination tool available for microbiota-related analysis of FFPE tissues, numerous low-biomass microbiota studies applied adapted decontamination frameworks (e.g., control-based subtraction, prevalence filtering) as well as common computational methods such as decontam or miroDecon [12, 14, 15]. Recently, Austin et al. reported 'Source-tracking for Contamination Removal in microBiomes (SCRuB)'—a method for high-precision decontamination of count-based microbial data using control samples, which was successfully applied to a study of the human tumour microbiome and thus might hold the potential to be used in microbiota analysis of FFPE tissues [16].

Current evidence further suggests that bacterial communities detected in FFPE tissues do not fully recapitulate those found in matched fresh-frozen samples, raising concerns about the biological relevance of FFPE-derived microbiome data [17]. Particularly, the variability in fixation durations (sometimes years), archival time, and extraction methods can skew microbial profiles compared to fresh/frozen tissues [18]. The feasibility of microbiome sequencing from FFPE tissue is therefore conditional and application-dependent. For studies requiring broad microbial community profiling, fresh-frozen specimens remain the gold standard. However, for targeted analyses of specific microbial markers, or when archived specimens are the only available resource, FFPE tissues may provide valuable but limited insights, particularly when nested PCR (Polymerase Chain Reaction) or qPCR (quantitative/real-time PCR) is used to enhance sensitivity for the targeted detection of microbes or associated virulence genes [13]. Moreover, pairing FFPE-based analysis with laser capture microdissection (LCM) or laser microdissection (LMD) for localisation of microbial communities within tissues before DNA extraction was recently successfully implemented and could offer new opportunities [19]. In addition, the combination of microbiome data with host genomic, transcriptomic, proteomic, and metabolomic data from the same FFPE samples has been proposed as a promising approach to enhance biological insights [20].

4 | Opportunities in Using Stored FFPE Samples

In some countries, such as the Scandinavian countries, archived routine-care collected FFPE samples provide access to large patient cohorts with long-term clinical follow-up data. Biopsies are often taken at clinically relevant time points, such as at diagnosis, during relapse, when assessing drug adverse effects, or when considering treatment changes or surgery. Consequently, biopsies taken before IBD diagnosis are representative of early pathophysiological events before any treatment has

been initiated. In contrast, biopsies taken during follow-up may be used to address specific research questions related to clinical situations, such as drug response, outcomes after surgery, drug adverse events etc. Such studies may address rare events that are typically underrepresented in clinical drug-development studies. Furthermore, about 10% of adult patients with IBD experience a change of diagnosis, which is captured in real-world data but often missed in prospective study baseline collections. Delineating disease trajectories through serial measurements offers another unique research opportunity to understand disease progression, identify critical transition points, and tailor interventions based on individual patient patterns over time. Finally, one study found that the vast majority of patients with IBD underwent biopsies during diagnosis and follow-up, which, if routinely performed, would support the generalizability and applicability of trial outcomes to real-world clinical practice [21].

5 | Considerations When Using Stored Biopsies for Research

All the above-mentioned methods are based on sections of biopsy tissue and build on traditional histological observations using routine staining (e.g., H&E (hematoxylin and eosin)) and antibody staining. It should not be underestimated how much information can be retrieved from those H&E-stained tissues, especially if the pathologist is assisted by artificial intelligence (AI)-based image analysis tools [22]. In this context, the development of robust histological scores for monitoring mucosal healing and predicting clinical outcomes is promising, particularly when they can be effectively incorporated into AI systems to enhance diagnostic accuracy and support more personalised therapeutic decisions [23].

As discussed in detail above, one of the main challenges associated with FFPE microbiome analysis is the limited DNA quality and length caused by depurination and cross-linking during formalin fixation [12] as well as fragmentation, which has been shown to increase with storage time. However, only a few alternative fixatives are available, and in general, none is superior to formalin. A recent, comprehensive study reviewed strategies for mitigating DNA damage [24]. Reversal of formaldehyde fixation for RNA studies is also an option, but it is unlikely to be practical for larger-scale studies [25].

Also, storage conditions may vary, for example, due to atmospheric conditions, and this, in turn, can influence the likelihood of deterioration [26]. The duration of storage of routine samples often varies between and even within countries. Outsourcing to a large archiving facility is common. Accordingly, the UK Royal College of Pathologists (https://www.rcpath.org/static/1111c528-c7ff-443d-9c3706f8acd19840/g031-draft-bpr-the-retention-and-storage-of-pathological-records-and-specimens.pdf) recommends the storage of tissue blocks (paraffin wax) for 30 years and of tissue sections and digital images for a minimum of 8 years.

Sampling protocols for IBD differ considerably between and within countries and even within a single institution. The European Crohn's and Colitis Organisation (ECCO) recommends that 'For a reliable diagnosis of ulcerative colitis, ileocolonoscopy should be performed. A minimum of two biopsies from each of the five sites in the colorectum and terminal ileum should be obtained. Additional biopsies should be taken from the endoscopically most severely affected area, specifically at the edge of ulcers if present' [27]. However, adherence to this recommendation is not widespread, and indeed, there may be other national and international recommendations that are not the same. Furthermore, protocols for and approaches to endoscopic sampling for IBD follow-up are even less consistent than those for suspected new IBD.

Methods for sending endoscopic biopsy samples from IBD patients to pathology laboratories also demonstrate considerable variation. Options include placing biopsies from all sites into one specimen container (generally regarded as poor practice), using a separate specimen pot for each site (allowing reliable identification of biopsy site and minimizing the risk of intermingling of samples but increasing the cost and the consumption of resources), and usage of multi-well containers or microcassettes (considerably more economical than separate pots but increasing the risk of mix-ups and reducing the number of fragments that can be submitted) [28]. Financial and environmental considerations often take priority over the desire to adopt the ideal approach. How best to address these inconsistencies is not yet obvious.

Clinical metadata is often limited, and information on disease activity, diet, lifestyle, and the microbiome is lacking. Many countries store information on anatomy and pathology for each FFPE sample, based on the SNOMED (Systematised Nomenclature of Medicine) International classification, including anatomical localisation (topography (T)) and morphological changes in tissue, cells, and other structures (morphology (M)). Some countries, including the Nordic countries, can link this information to National Health Service registries, providing details on diagnosis codes, hospital contacts, medications, surgeries, and results from laboratory tests, etc. [21].

The disease location may also affect the use of FFPE biopsies in research. Endoscopically sampled gut biopsies may better reflect the disease mechanisms of ulcerative colitis than the patchy, transmural changes of Crohn's disease, in which biopsies may not accurately reflect clinically relevant changes occurring in layers beneath the mucosa.

Finally, the feasibility of retrieving the biopsies from physical storage should also be considered.

To address variation in study conditions, studies may provide details on a range of pre-analytic factors, including procurement, fixation, processing, and storage. Recommendations for the minimal essential technical information to be provided in a scientific manuscript have been proposed [24].

In many countries, ethical regulations govern the use of routine care-collected material for research. The UK Human Tissue Act regulates activities concerning the removal, storage, use and disposal of human tissue in England, Wales and Northern Ireland (https://www.hta.gov.uk/guidance-professionals/hta-legislation).

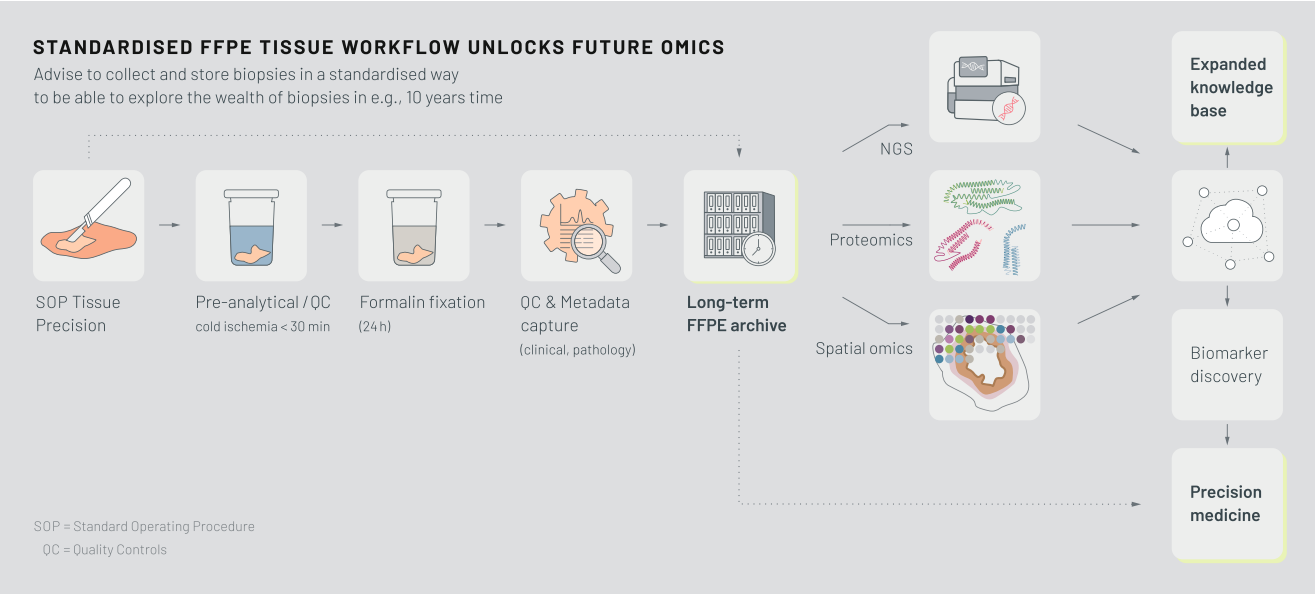

FIGURE 2 | Standardised FFPE tissue workflow for unlocking future omics and advancing precision medicine. This figure outlines the critical need for standardised protocols in IBD care, covering sample procurement, fixation, processing, and archiving, to enable high-quality omics research necessary for biomarker discovery and personalised treatment strategies.

6 | Unlocking the Precision Medicine Potential of Clinically Collected Gut Biopsies

From an ethical perspective, there is a responsibility to utilise available resources to improve patient outcomes. Based on this and the discussion above, we strongly recommend developing guidelines for IBD routine care biopsy management (Figure 2). Standardised methods for routine clinical biopsy collection and storage are already in place in some areas, such as cancer treatment (https://www.rbgb.dk/en/om-rbgb/). Adopting standardisation procedures similar to those in IBD care would promote high-quality research, enhance equity by including more diverse patient populations, deepen our understanding of disease mechanisms, and facilitate the identification of clinically relevant biomarkers.

In conclusion, despite the high potential of archived routine-care biopsy material for research, the heterogeneity of biopsy collection and storage methods continues to hamper its widespread use. FFPE tissues hold significant promise for molecular research, particularly for the retrospective delineation of disease trajectories through serial measurements and spatially resolved studies. Overcoming methodological challenges and establishing standardised processes for collecting and storing routine care biopsies—similar to practices already implemented in some countries for cancer—is essential to gain new insights into IBD.

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Conflicts of Interest

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Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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