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- Research and Innovation Action -

# D4.1
# Obtaining tissue

WP 4 – Good Manufacturing Practice (GMP) processing and scaling up

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Lead beneficiary for this deliverable: UCL

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# History table

| Version | Date | Released by | Comments |
|---|---|---|---|
| 1 | 14.02.2017 | Paolo De Coppi | |

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# 1. Introduction

This document reports on “Deliverable 4.1: Obtaining tissue” derived from work within Work Package 4 “Good Manufacturing Practice (GMP) processing and scaling up”.

In order to generate a tissue engineered intestine, we need to isolate and expand the cell populations that exist in the adult human intestine, which together form the complete organ. Using primary human biopsies, we can develop robust methods for the isolation and expansion of the necessary cell populations.

In order to harvest primary human tissue, ethical approval must be sought and granted.

The aim of this deliverable is to show that we have obtained regulatory approval and to define logistics and quality control measures needed for a sample to be processed for in vitro culture and graft development.

# 2. Summary of activities and research findings

The research team at UCL has in place ethical approval for the harvesting of primary human intestinal tissue from eligible patients at Great Ormond Street Hospital NHS Foundation Trust. NHS Ethics Ref 04/Q0508/79.

Patients who meet the inclusion criteria for this study are identified and invited to participate (consent process). Patients who are Gram positive are excluded from the study. Endoscopic biopsies are obtained from routine procedures, full thickness gut samples are removed during surgery. Endoscopic biopsies are maintained in sterile PBS while full thickness samples are placed on dry gauze until collected for laboratory processing. Samples are processed within a maximum of one hour from collection.

Tissue samples are washed a minimum of three times in media containing antibiotics to remove residual digested or faecal matter. Samples from patients with inflammation are subject to three extra washes. Biopsies from patients with severe intestinal disease can disintegrate at this stage. If this occurs, samples are abandoned as previous experience has shown that tissue dissociation at this stage is associated with poor cell viability and low in vitro clonogenic potential.

The postnatal gut biopsy specimens which remain intact following initial washes are subjected to mechanical and enzymatic dissociation according to the protocol published by *Metzger et al. 2009.* Organoids are cultured from these biopsies.

# 3. Conclusions and future steps

The protocol is currently being optimized to determine the number of washes required before mechanical dissociation, and the optimal length of time for enzymatic digestion in order to maximize yield for downstream processes.

## 4. Bibliographical references

*Marco Metzger, Amanda J. Barlow, Alan J. Burns, Nikhil Thapar (2009) Enteric Nervous System Stem Cells Derived From Human Gut Mucosa for the Treatment of Aganglionic Gut Disorders. Gastroenterology, 136 (7) 2214–2225.e3.*