Exploration of the roles of N0, N1, and N2 neutrophils on bone and cartilage regeneration in vitro from blood samples drawn from healthy volunteers

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Primary Objective: The objective of this trial is to examine the distinctive surface biomarkers and cytokines found in the medium of N1 and N2 neutrophils. Furthermore, this study aims to investigate the varying effects of N1 and N2 neutrophils on...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Bone disorders (excl congenital and fractures)
Study type	Observational invasive

Summary

ID

NL-OMON56745

Source
ToetsingOnline

Brief title
Neutrophils and Bone/Cartilage Regeneration

Condition

- Bone disorders (excl congenital and fractures)

Synonym
Cartilage damage, Fracture healing

Research involving
Human

Sponsors and support

Primary sponsor: Medisch Universitair Ziekenhuis Maastricht

Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: Bone regeneration, Cartilage regeneration, Neutrophils

Outcome measures

Primary outcome

The main study parameter involves the unique surface biomarkers and cytokines present in the medium of N1 and N2 neutrophils, which will be assessed using flow cytometry and ELISA. Additionally, the diverse stimulatory effects of N0, N1, and N2 neutrophils on the osteogenesis of BMSCs and the chondrogenesis of chondrocytes will be investigated through presto blue, ALP activity, qPCR, as well as alizarin red and alcian blue staining.

Secondary outcome

The metabolites produced by N1 and N2 neutrophils and their spatial distribution will be examined using MALDI-MSI.

Study description

Background summary

Neutrophils, comprising 60-70% of white blood cells, play a pivotal role as the predominant cell type in human blood and are a key component of the innate immune system. Functioning as the frontline defenders against PAMPs and DAMPs, neutrophils migrate to inflammatory sites by deformation, adhesion, infiltration, and migration. Subsequently, neutrophils carry out essential functions through various mechanisms, such as the formation of NETs and the production of cytokines and granular proteins.
Nonetheless, an expanding body of research is revealing the diverse functions of neutrophils, moving beyond their singular roles and attempting to uncover their potential mechanisms. A notable example is the work of Fridlender et al., who initially proposed the N1 (pro-inflammatory) and N2 (anti-inflammatory) neutrophil phenotypes based on their distinct functions in

cancer, drawing an analogy with the M1 and M2 macrophage phenotypes. Subsequently, the "N1/N2" terminology gained widespread usage in various fields, including cardiology, clinical immunology, neurology, and infectious diseases, to denote the multifaceted effects of neutrophils. Building on this understanding, Ohms et al. developed a protocol to polarize human neutrophils into N1 and N2 phenotypes in vitro, facilitating further exploration of the characteristics of N1 and N2 neutrophils. In subsequent studies, researchers have employed similar methods to identify distinct neutrophil phenotypes based on their surface biomarkers. Neutrophil subsets, designated as N1 (characterized by CD54 and CD95) and N2 (defined by CD182), were thoroughly investigated. These investigations have unveiled the diverse roles played by these distinct neutrophil phenotypes across a wide array of fields.
Bone and cartilage healing is an intricate process involving the active participation of neutrophils. The manifold roles of neutrophils in bone regeneration have been widely acknowledged, encompassing diverse mechanisms such as the formation of NETs, modulation of angiogenesis, influence on the extracellular matrix, and interaction with macrophages, among others. Nevertheless, there is a paucity of studies linking varied effects of neutrophils with N1/N2 neutrophil phenotypes. Cai et al. have elucidated the beneficial role of N2 neutrophils in mouse bone healing, highlighting the involvement of Stromal cell-Derived Factor 1 (SDF-1)/CXCR4 signaling. However, it remains unclear whether N1 and N2 neutrophil phenotypes can be attributed to the diverse roles observed in human bone regeneration. Furthermore, the recognition of N1 and N2 neutrophils is still an empty in the field of cartilage regeneration.
In this study, we utilize the polarization of N1 and N2 neutrophils to explore potential differential effects on the osteogenesis of human BMSCs and the chondrogenesis of chondrocytes. We hypothesize that N1 neutrophils inhibit, while N2 neutrophils promote both osteogenesis and chondrogenesis.

Study objective

Primary Objective: The objective of this trial is to examine the distinctive surface biomarkers and cytokines found in the medium of N1 and N2 neutrophils. Furthermore, this study aims to investigate the varying effects of N1 and N2 neutrophils on bone and cartilage regeneration. We hypothesize that N1 neutrophils display inflammatory properties (elevated levels of CD54, CD95, Tumour Necrosis Factor α (TNF-α), IL-8, Monocyte Chemoattractant Protein 1 (MCP-1)), leading to the inhibition of bone and cartilage regeneration. Conversely, we anticipate that N2 neutrophils exhibit anti-inflammatory characteristics (elevated levels of CD182, SDF-1α), thereby promoting bone and cartilage regeneration.

Study design

Our study 'The effect of N1-N2 neutrophils on bone/cartilage regeneration':

This study is an in vitro study in which blood will be drawn from healthy control volunteers by venipuncture. Neutrophils will be isolated from healthy donor blood in the laboratory and stimulated in vitro using a specialized culture medium. Following stimulation, the neutrophils anticipated to exhibit the N0, N1, and N2 phenotypes will be examined by flow cytometry and ELISA technique, subsequently directed to stimulate BMSCs or chondrocytes. Subsequently, the osteogenic and chondrogenic potential of BMSCs and chondrocytes, respectively, will be systematically analyzed. Furthermore, N0, N1, and N2 neutrophils will artificially adhere to slides for MALDI-MSI to analyze the unique metabolites and their spatial distribution associated with the N0, N1, and N2 phenotypes.

Study burden and risks

The venipuncture will be done by competent and authorized technicians (according to the Dutch law, *voorbehouden handeling, wet BIG* and the NVKC guideline *veneuze bloedafname*). Blood sample collection is a relatively minor invasive procedure, from which we do not foresee complications other than the formation of small hematomas or minor hemorrhage at the site of venipuncture. Subjects do not have to be sober at the time of blood sample collection. Therefore, we anticipate only minor discomfort as a consequence of participation. Our proposed research will not directly benefit the subjects included in our study. However, our study can lead to new insights from which patients may benefit in the future.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)

Inclusion criteria

Healthy men and women, aged > 18 years
Signed written consent to take part in the study

Exclusion criteria

Current infection, such as: cold, flu, sore throat, cold sores,
gastrointestinal infections, or any other infections.
Recent tattoo or body piercing within last six months
Minor dental surgery within 24 hours, or major dental surgery within one month
Recent anemia.
Travel to areas where mosquito-borne infections diseases are prevalent, such as
malaria, dengue fever, and Zika virus infection
Positive for HIV
Ever injected recreational drugs
Pregnancy and breastfeeding
Have a history of cancer

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	01-07-2024
Enrollment:	20
Type:	Actual

Ethics review

Approved WMO

Date:	21-05-2024
Application type:	First submission
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL86025.068.24