Medicinal Signaling Cells Metabolite Oral Based as a Potential Biocompatible Biomaterial Accelerating Oral Ulcer Healing (*In Vitro* Study)

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Abstract

Objective Medicinal signaling cells metabolite (MSCM) is often considered medical waste even though it contains abundant growth factors, and advantageous micro- and macromolecules that can accelerate healing in oral ulcer.
The purpose of this experimental laboratory study was to analyze the biocompatibility and potential of MSCM, (oral based) to accelerate healing in oral ulcer (in vitro).
Materials and Methods MSCM (oral based) was obtained by mixing 10 mL of MSCM and 2% of carboxymethyl cellulose sodium. 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (or MTT assay) was obtained using human gingival somatic cell culture to examine cell viability treated with MSCM (oral based). Fourier transform infrared spectroscopy was performed to know the functional structure and composition of MSCM (oral based). To know the elemental composition of MSCM (oral based), energy-dispersive X-ray analysis was performed. Scratch test was performed to know the ability of MSCM (oral based) to increase human somatic cell proliferation.
Results MSCM (oral based) has good cell viability. MSCM (oral based) administration accelerated the proliferation of human somatic cell culture after 12-hours in vitro. MSCM (oral based) has carboxylic acids and derivatives chemical bond. MSCM (oral based) mostly contained carbon and potassium but did not contain heavy metal substances.
Conclusions MSCM (oral based) has a biocompatible and potential ability to accelerate healing in oral ulcer in vitro. It would be useful in daily clinical practice in treating traumatic oral ulcer.

Keywords
- medicinal signaling cells
- wound healing
- biomaterial
- metabolite
- biocompatibility

Introduction

Sharp orthodontic appliances can lead to oral tissue injury. Traumatic oral ulcers are frequently related to sharp orthodontic brackets or wires that irritate the oral mucosa. The destruction of the oral epithelium is marked by irregular laceration or ulcer surrounding the erythema area and nerve ending exposure. Traumatic oral ulcers caused by sharp

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orthodontic appliances can cause unpleasant and painful sensation.[1] According to the Khan et al study, oral mucosa lesions—such as 40% patients with erosion and 45% patients with ulceration—frequently occur during fixed orthodontic treatment.[2] Traumatic oral ulcer incidences range from 60 to 81%. An estimated 47% traumatic oral ulcer incidences occurred in adults with the most unpleasant sensation during orthodontic treatment; 29% of adolescents report ulcers as the second-most discomfort aspect of orthodontic treatment.[1,3]

Painful sensation is part of the inflammatory response during healing that occurs ~24 hours.[4] Oral ulcers normally heal in ~10 to 14 days, but sometimes if there is infection, it can prolong and delay the healing process.[1,4] The infection stimulates pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β. Those pro-inflammatory cytokines stimulate the synthesis of matrix metalloprotease-9, which can lead to Extracellular Matrix degradation.[5] There are so many normal commensal flora in the oral cavity. Although saliva has antimicrobial properties like human beta defensin-2 as antimicrobial peptide (AMPs), the microenvironment in the oral mucosa is very challenging for oral ulcer healing due to pathogen microorganisms in the oral cavity that can induce an inflammatory response.[6,7]

So many products and drugs, with different formulations and various active compounds, were used to treat oral ulcers.[8,9] The Karavana Hizarcioğlu et al study mentioned that chlorhexidine or benzydamine hydrochloride is no better than a placebo mouthwash. Ulcers treated with benzydamine hydrochloride gel reduced 33% more than ulcers in control groups, but they were still not healed until the 12th day.[9] Ernawati and Puspa and Puspasari et al studies used herbal compounds such as propolis gel to treat oral ulcers, while an Apriasari et al study used mauli banana stem extract gel as an anti-inflammatory agent to treat oral ulcers, but the healing process accelerated after a few days.[4,5,10]

Medicinal signaling cells or mesenchymal stem cells (MSC) possessed the ability to accelerate wound healing.[11] During MSC culture in medium, MSC secreted a lot of beneficial cytokines, AMPs, molecules, and growth factors in the culture medium that were very useful.[12,13] After MSC was harvested, the MSC culture medium was discarded and become medical waste. The MSC culture medium can be purified and can be used again as biomaterial to accelerate oral ulcer healing.[14]

We would like to make the promising biomaterial, which has great biocompatibility and potential ability to accelerate oral ulcer healing by MSCM (oral based). The aim of this study was to analyze the analyze the biocompatibility and potential of MSCM (oral based) accelerating oral ulcer healing (*in vitro*). This is important and useful because traumatic oral ulcer is a major problem during fixed orthodontic treatment that has yet to be resolved.

Materials and Methods

This study was conducted in an experimental laboratory with a descriptive analysis observational study and a randomized control group design. The study received ethical clearance with number 289/HRECC.FODM/XII/2017 from Faculty of Dental Medicine, Universitas Airlangga, Surabaya.

Formulation of Medicinal Signaling Cells Metabolite (Oral Based)

The MSCM (oral based) gel was made in accordance with the procedures of the Stem Cell Research and Development Center by mixing 10 ml of MSC metabolite with 2% of carboxymethyl cellulose sodium (►**Fig. 1**).[14]

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide or MTT assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (or MTT assay) was performed using human gingival somatic cell (GSC) culture in accordance with the procedure prescribed in the Prahasanti et al study.[15] GSCs were isolated and cultured according to the procedure prescribed in the Nugraha et al study.[16-18]

Furthermore, the samples were divided into two groups: cells treated with MSCM (oral based) were the treatment group and cells without MSCM (oral based) were the control group. The number of living cells was then observed by calculating CD50 with the MTT assay. MTT absorbency was indicated with a crystal formazan blue purple colored was examined by means of enzyme-linked immunosorbent assay reader using 550 to 620 nm wavelength. The MTT assay was done at the Stem Cell Research and Development Center, Surabaya, Indonesia.

Scratch Test

A scratch test was done at the Stem Cell Research and Development Center, Surabaya, Indonesia. The scratch test was performed to know the ability of MSCM (oral based) to stimulate human somatic cell proliferation and migration, assessed with cell migration into the cell-free field, in accordance with the Rantam study.[14] Human somatic cells were cultured in an m24 culture plate with estimated 5.10^4 cells in each well, then incubated at 37°C in an incubator (Sigma Aldrich, US). Each well was scratched with blue tip (Sigma Aldrich, US) with plus (+) shape. The samples were divided into four groups (n = 1): A. Control group; B. 0 hour treated with MSCM (oral based); C. 3 hours treated with MSCM (oral based); D. 12 hours treated with MSCM (oral based). The culture plate of each group was examined by means of a light microscope with 100x magnification (Olympus, Japan). Images were taken with a camera (Olympus, Japan).

Fourier-Transform Infrared Spectroscopy and Energy Dispersive X-Ray Analysis

The MSCM (oral based) product was examined by means of Fourier transform infrared spectroscopy (FTIR) to know its functional structure and composition, and to know the elemental composition of MSCM (oral based), an energy dispersive X-ray (EDX) analysis was performed. The EDX and FTIR analyses were done accordance with the procedure of the Prahasanti et al and Nugraha et al studies.[15,16] Both analyses was done at the Laboratory of Technical Industrial Faculty, Institute of Technology Sepuluh Nopember, Surabaya, Indonesia.

Results

The optical density of MSCM (oral based) can be seen in ►**Fig. 2**. The MTT assay examination result showed that MSCM (oral based) has a high viability to human somatic cell culture (►**Fig. 3**). The scratch test result showed that MSCM (oral based) administration accelerated the proliferation of somatic cell culture after 12 hours in vitro (►**Fig. 4**). The FTIR examination showed a functional structure and composition of MSCM (oral based) that can be seen in ►**Fig. 5**. It has Carboxylic Acids & Derivatives bond with peak ~3270.11. The EDX examination showed that MSCM (oral based) mostly contained Carbon Potassium and it did not contain heavy metal substances (►**Fig. 6**). The composition of MSCM (oral based) can be seen in ►**Table 1**.

Fig. 1 Medicinal signaling cells metabolite (oral-based) product.

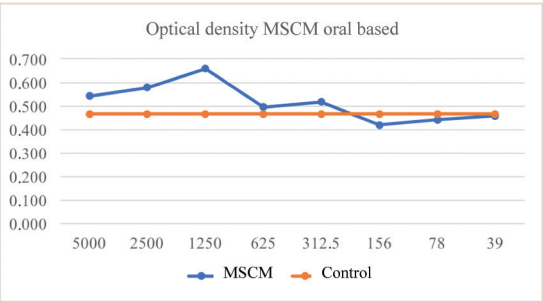

Fig. 2 Optical density of medicinal signaling cells metabolite (MSCM) (oral based).

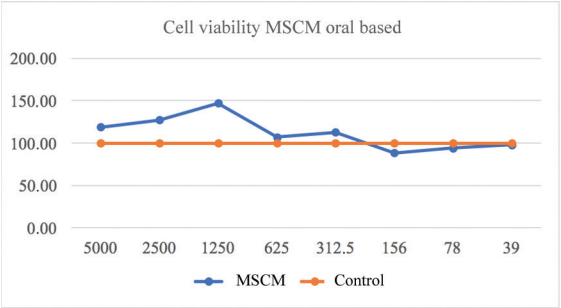

Fig. 3 Cell viability of medicinal signaling cells metabolite (MSCM) (oral based).

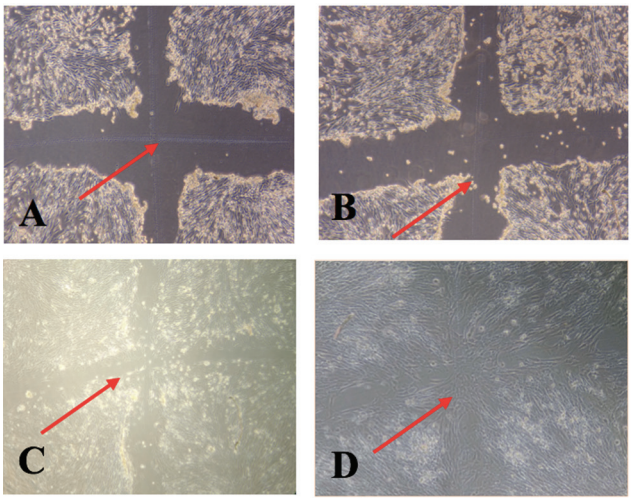

Fig. 4 Scratch test to examine somatic cell proliferation ability (in vitro) examined using electron microscope with 100x magnification (Olympus, Japan). (**A**) Control group, (**B**) 0 hour treated with medicinal signaling cells metabolite (MSCM) (oral based); (**C**) 3 hours treated with MSCM oral-based product; (**D**) 12 hours treated with MSCM (oral based).

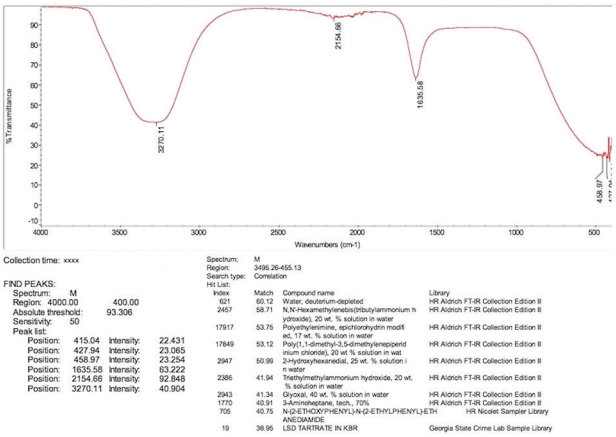

Fig. 5 Fourier-transform infrared spectroscopy result of medicinal signaling cells metabolite oral-based functional structure and composition.

Discussion

The successfulness oral ulcer treatment depends on several factors such as the elimination of etiology factor, minimalize

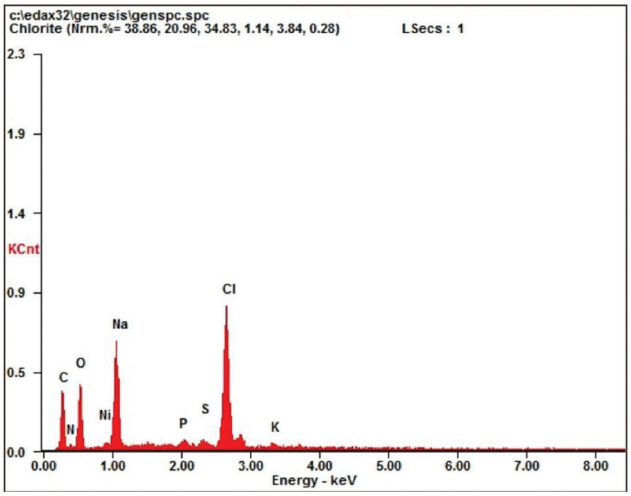

Fig. 6 Energy-dispersive X-ray result of medicinal signaling cells metabolite oral-based product.

Table 1 Element composition analysis using EDX of MSCM oral based

Element	Wt%	At%
C K	28.06	40.81
N K	04.40	05.49
O K	26.59	29.04
Ni L	01.19	00.35
Na K	17.44	13.25
P K	00.86	00.48
S K	00.87	00.47
Cl K	19.67	09.69
K K	00.92	00.41

Abbreviations: EDX, energy-dispersive X-ray; MSCM, medicinal signaling cells metabolite.

the pathogen or commensal microorganism in the oral niche that can delay healing.[4,5,10] A biocompatible biomaterial that supports cell, and the potential growth factor in biomaterial to support endogenous cell metabolic activities is necessary needed.[17-19] MSCM (oral based) has good cell viability in GSCs examined by the MTT assay. GSCs can be easily isolated with noninvasive methods from free-margin gingiva in the oral cavity.[20] GSCs are suitable cells for cytotoxicity test purpose.[21] It can be said that MSCM (oral base) is a biocompatible material for application in the oral cavity. Surprisingly, MSCM (oral based) did not decrease the cell number during the MTT assay but increased it. MSCs during culture secreted abundant advantageous growth factors through paracrine and autocrine pathways such as vascular endothelial growth factor, fibroblast growth factor, and insulin growth factor, which support cell metabolism, proliferation, and differentiation.[22-24] The scratch test support the fact that growth factor contained in MSCM (oral based) can increase cell proliferation after 12 hours. MSCM (oral based) stimulates migration factors such as CXC motif chemokine ligand 12 and stromal-derived factor-1 that made communication of the cells happen. In addition, cells secreted various growth factors to support cell proliferation.[24-27] The Somoza et al study also mentioned that MSCs secreted antimicrobial peptides such as Cathelicidin against microorganism and Immunomodulation molecules like Indoleamine 2,3-dioxygenase.[12] Those beneficial molecules are very useful for tissue regeneration caused by injury. At the injury sites, MSCs focus their local immunomodulatory and trophic activities.[24-27]

The FITR results of this study revealed the function and structure of MSCM (oral based). FTIR spectroscopy will result in a powerful tool in the study and diagnosis of biological systems. FTIR spectroscopy is a rapid, noninvasive, accurate, and efficient technique to examine biomaterial.[15] The structural organization of MSCM (oral based) is an important matrix for retaining the cells at a specific site and initiating appropriate cell-to-cell interactions and migration.[22]

EDX is very useful in the biomedical fields because of its high sensitivity to examine the different elements in tissues. EDX is generally used to improve the biomaterial or chemotherapeutic agents. EDX is also an important tool to detect nanoparticles.[28] In this study, EDX was performed to examine the composition of MSCM (oral based). MSCM (oral based) was a safe biomaterial because it did not contain heavy metal substances such as lead (Pb), nickel (Ni), zinc (Zn), cadmium (Cd), chromium (Cr), copper (Cu), or mercury (Hg). EDX can be used to detect heavy metal substances due to pollution in the environmental. Heavy metals are nonbiodegradable substances. These are the biggest problem for the environment, and they can accumulate and negatively affect the human body.[29]

Conclusion

MSCM (oral based) has a biocompatible and potential ability to accelerate healing in oral ulcer in vitro. It would be useful in daily clinical practice in treating traumatic oral ulcer.

Conflict of Interest
None declared.

Acknowledgments
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