



**SEEDING OF MESENCHYMAL SHED ABILITY IN FREEZE DRIED AMNIOTIC MEMBRANE WITH PRP COATING IN HUMAN BLOOD PLASMA MEDIA**

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**SUMMARY**

**Introduction:** The aims of this research to determine the number of mesenchymal SHED attached to freeze dried amniotic membrane which cultured in human blood plasma supplemented medium.

**Material:** Mesenchymal SHED, freeze dried amniotic membrane, human blood plasma, FBS, alpha MEM medium, PBS, triple express and collagenase-1 enzymes.

**Method:** Monolayer cells seeding with a concentration of  $2 \times 10^6$  on a 2x2 cm freeze dried amniotic membrane. The sample used divided into two treatment groups. The first group was MSC seeding on a freeze dried amniotic membrane that coated with PRP and cultured in a human blood plasma supplemented medium. The second group was control by cultured MSC in amniotic membrane without PRP coated then culture in FBS supplemented medium. Mesenchymal SHED was seeded on amniotic membrane then incubated for 1 day, 3 days, and 5 days. At the end of the incubation time, each treatment was calculate the number of cells attached to the amniotic membrane surface with a cell counter. The data collected analyzed by Anova One Way statistic test with ( $P < 0,05$ ).

**Result:**

Table 1. The average of the number cells was seeding on freeze dried amniotic membrane each group at various incubation times

Treatment	Incubation time		
	1 day	3 days	5 days
Human Blood Plasma Supplemented medium	5508,75 ± 378,08*	6544,25 ± 245,53	6756,5 ± 764,38
FBS Supplemented medium	5182,25 ± 180,32*	6698,75 ± 285,80	6514,75 ± 142,13

Results from statistical analysis with ( $P < 0.05$ ) revealed that the ability of treatment groups compared with control showed significant difference on day 1 and did't showed significant difference on the 3rd and 5th days.

**Discussion:** The results of this research the treatment groups were significantly different on day 1 because mesenchymal SHED became more active with growth factor IGF-2 and CDK-6. Blood plasma supplemented medium contains many natural growth factors such as TGF- $\beta$  that function in the process of proliferation MSC and the accumulation of ECM in the form of laminin and fibronectin for interaction between cells as an ECM composite that can help cells attach to the amnion membrane surface (Rantam et al, 2014) the number of cells on days 3 and 5 did't show a significant difference due to the availability of nutrients in the culture medium was thinning so the cell degenerate and become damaged. The accumulation of toxic cell secretions such as trypsin which is a cell proliferation inhibitor by loosening the extracellular matrix bonds that can release cells from the surface of the amniotic membrane. Another factor that plays a role is low (IGFBP-7) expressed by DPSCs, known to bind to IGF-1 and IGF-2 which are factors that can inhibit cell growth (Turksen, 2004).

**Conclusion:** The number of mesenchymal SHED seeding on freeze dried amniotic membrane with coating PRP more than control groups.