ABSTRACT

Partial/superficial thickness wounds frequently seen in clinical practice, whereas in major burns lead to the devastating effects. The freeze-drying method for amnion preservation was mostly acceptable and became standard method in Dr. Soetomo Biomaterial Centre and Tissue Bank Surabaya with sterile condition granted by Gamma irradiation. Unfortunately, several research had shown no superiority of freeze-dried amnion (FD) compared with other modalities. These due to significant decreased amount of growth factors. Recently revealed that FD contain of High Molecular Weight Hyaluronate which contributed to wound healing by its natural degradation into Low Molecular Weight. Addition of Low Molecular Weight Hyaluronate (LMWH) were expected to enhance wound healing by FD. Twenty four Wistar rats (Rattus norvegicus) were wounded superficially on the back torso in 3 locations of each. One was covered with fresh amnion (FA Group), one covered with freeze-dried amnion (FD Group), the other covered with freeze-dried amnion + LMWHA 1% (HA Group). Samples collected randomly at day 1, 3, 5 and 7 with 6 rats sacrificed at once. Histological changes were observed for the amount of epithelial layer, epithelial thickness and maturation. Data were distributive analyzed by Kolmogorov-Smirnov and colleration analyzed by Anova and Kruskal-Wallis test. No epithelization demonstrated at day 1 in all groups. HA group had epithelization rate more inferior than FA group in day 3, but showed superiority in day 5 and 7 (p<0,05). FA group had epithelization rate more superior than FD group in day 3,5 and 7 (p<0,05). FA group had epithelization rate more superior than FD group in day 3 and 7 (p<0,05) but not in day 5 (p>0,05). FA group showed better in epithelial maturation compared in two other groups but there’s not significant (p>0,05). (FMI 2013;49:229-236)

Keywords: LMWHA, Fresh amnion, Freeze-dried amnion, growth factors, enhance wound healing
INTRODUCTION

Superficial wounds that do not reach the entire thickness of the dermis (Tredget et al 2006, Young 2006) are common in the treatment of surgical cases, especially in excoriated wounds, second degree burns and superficial wounds on the donor Split Thickness Skin Graft. Superficial wound is expected to experience a period of time until epithelization at 10-14 days (Young 2006). Wound care Split Thickness Skin Graft Donor becomes very important in the case of the limited availability of donors to cover exposed skin in extensive burns. It is expected that donor skin that has undergone epithelization can be a source of new donors for closure (Young 2006). In the treatment of superficial-degree burns are also expected that epithelization happen as soon as possible to avoid the complications of local and systemic infection and immediately stop the ongoing process of hypermetabolism (Young 2006, Heimbach Heimbach & Bozarth 2006).

Several studies have shown that the use of paraffin gauze is the most inferior in epithelization spur, can cause trauma to the newly formed epithelium at the time of removing the bandage and less comfortable for patients because of pain when changing dressings (Saputro & Noer 2001, Padmani & Perdanakusuma 2008). Calcium Alginate is said to stimulate the wound superficial velocity exceeds epithelization hemicellulose and tulle but equal with preserved amnion (Padmani & Perdanakusuma 2008). There are some researchers say fresh amniotic far have advantages due to the nature of biological dressings that have an anti-bacterial effect, reducing pain and contains growth factors that stimulate wound healing (Gruss & Jirsch 1978, Talmi et al 1990, Koizumi et al 2000, Saputro & Noer 2001, Wolbank et al 2009).

Amniotic membrane used is preserved amniotic membrane (preservation) is freeze-dried. This is because fresh amniotic membrane eligible free of hepatitis, HIV and Syphilis can not be obtained at any time, so it is more practical when using amniotic membrane that is preserved. Freeze-dried preservation techniques has been based on international standards include the sterility. Price preserved amniotic membrane is also relatively affordable. Hyaluronic acid is a glycosaminoglycan component of the extracellular matrix that plays a role in the wound healing process and is produced by fibroblast cells (Jenkins et al 2005). The content of hyaluronic is also found in large numbers on fresh amniotic membrane proved to make the amniotic membrane has anti-inflammatory properties and simultaneously modulating angiogenesis. The hyaluronic levels far lower in the preserved amniotic membrane (Shay et al 2009) hyaluronic consists of two groups: High Molecular Weight Hyaluronate or Hyaluronic Macromolecules (HAM) and its degradation results in the form of Low Molecular Weight Hyaluronate (Hyaluronic LMW), where the shown to stimulate angiogenesis LMW types, mitosis and cell migration of keratinocytes, fibroblasts and endothelial cells (Hamann et al 1995, Fraser et al 1997, West & Fan 2002, Gomes et al 2004). LMW hyaluronic also proved to spur growth factor production by macrophages and modulates the inflammatory response in the wound healing process. Several studies have shown LMW hyaluronic epithelization speed up the process compared with the control (King et al 1991, Chung et al 1998, West & Fan 2002). Applications freeze-dried amniotic membrane and hyaluronic LMW on superficial wounds are expected to support each other in the process of wound healing and accelerates the process of epithelization equivalent of fresh amniotic membrane.

MATERIALS AND METHODS

Freeze-dried amnion membranes was amnion which was produced by the Center Biomaterials/Network Bank Dr. Soetomo Surabaya that comes from human placenta that had fulfilled the requirement and had preserved with freeze-drying processes and sterilization techniques with the radiance according to standards set by the American Association of Tissue Bank (AATB). Fresh amnion was amnion membranes that bought directly from human placenta in fresh condition, has not been preserved and fulfill the requirement that is normal uncolded meconium and has gone through stages standard by the Bank Network Dr. Soetomo Hospital Surabaya that was washing with a Na Hipoklorit 0.05 percent and NaCl 0.9 percent and stored in the temperature of 4 degrees Celsius. Low Molecular Weight Hyaluronate was Hyaluronat as sodium Hyaluronat with form a sterile aqueous uncolored in PBS (Phosphat Buffered Saline 0.9 percent) with 1 percent concentration and a molecular weight under 500 kda. Low Molecular Weight Hyaluronate has been produced by Bioland Ltd. Songjeong, South Korea.

It has chosen 24 rats bull Rattus norvegicus strain Wistar healthy aged 40-60-day with a heavy 200-300 grams randomly. Be numbered 1-24 randomly. Rats were injected with Ketamine HCl 20-40 mg per kg weight intra muscular. Each rats had been shaved off its feathers at the back part, made design 3 square wound each measuring 1.5 x 1.5 cm to 2 in each of them right back as far as separate 2 cm and 1 on their backs left with spidol. After disinfection with solution Betadyn 10% resemblances very seriously an injuring made with the excision biopsy tangential wearing a Humby small
knife that wound square on that three design. The wound then compressed in adrenaline Solution in saline concentration 1:200000 U for 2 minutes to stop the bleeding as an oozing form that took place.

Injuries to their right backs close one wear amnion freeze processes-dried while the other with amnion fresh. Injuries to their left backs smeared with a Low Molecular Weight Hyaluronate 1 percent in the wound and then closed with amnion freeze-dried. In the three wounds that have been closed then closed again with a thick kassa that have been chosen to match with stitches using silk 4/0 on their rat’s back. All rats given injection Penicillin Procain intramuscularly 100mg/kg/day, as much as one-time and Mefenamic acid syrup with the dose 27 mg/day after it was good awake for the last 3 days. Rats are kept in their respective where its size and room also given food and types of a similar number according to standard AAALAC (Association for Assessment and Accreditation for Laboratory Animal Care). Wounded observed day 1, 3rd, 5th and 7th post injury in histopathological where each observation was selected 6 male rats at random and having been sacrificed and taken tissue incurable approximately to 0.5 cm outside the wounded included healthy tissue to examination histopathological. Wound tissue material folded with filter paper. Then it was given codes and be chosen to fixed with formalin solution 10 percent. The histopathology examination was done with routine Hematoxyline-Eosin coloring by The Anatomical Pathology Division.

Parameter that was tested is thick layer epithelial cells, the number of epithelial and epidermis maturity between these three groups at the same time and different from the day 1, 3rd, 5th and 7th. Result of the measurement thick layer epithelial cells and the Number of epithelial will be tested distribution and test Kolmogorov-Smirnov continued analysis of statistics with test Anova if the data are normally distributed, and Kruskal-Wallis if the data is not distributed normally. Result of the measurement maturity will be analysis of statistics with test Kruskal-Wallis. High significance on research that used was 0.05.

RESULTS

Total layer epithelial formed in the day 3 in the current amnion fresh (5.00±0.60 cell layers) and amnion freeze-dried + LMWHA (4.89±0.62 cell layers) superior to in the current amnion freeze-dried with total layer is less the cell layers 3.44±0.27 (p<0.0001). In the current fresh amnion is not different means with the group amnion freeze-dried + LMWHA (p>0.05).

On closer examination day 5th groups amnion freeze-dried + LMWHA epithelial cells have a number of layer that more but not different means the cell layers 0.35 6.50±group that fresh amnion (5.83±0.35) and the amnion freeze-dried (5.89±0.66) with the p=0.051. While between groups amnion fresh and groups amnion freeze-dried also did not significantly (p>0.05) so that the observation day 5th of the three groups not found difference between the numbers of cell layers. On closer examination day-7 group amnion freeze-dried + LMWHA epithelial cells have a number of layer 10.50±0.51 far surpassed the amnion fresh (8.33±1.46) and the amnion freeze-dried (7.61±0.44) with p<0.0001. While between groups amnion fresh and amnion freeze-dried are no different from means (Figure 1; Table 1).

Table 1. Difference between the numbers of epithelial layer on observation day 3, 5 and 7 between groups treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Amnion fresh</th>
<th>Amnion freeze processes and dried</th>
<th>Amnion Freeze Processes-dried + LMWHA</th>
<th>Rates p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total layer epithelium Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.00±0.60a</td>
<td>3.44±0.27a</td>
<td>4.89±0.62a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5</td>
<td>5.83±0.35a</td>
<td>5.89±0.66a</td>
<td>6.50±0.35b</td>
<td>0.051</td>
</tr>
<tr>
<td>7</td>
<td>8.33±1.46a</td>
<td>7.61±0.44a</td>
<td>10.50±0.51b</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 2. Difference between the numbers of epithelial layer in each group treatment between time watching the 3.5 and 7

<table>
<thead>
<tr>
<th>Group</th>
<th>Amnion fresh</th>
<th>Amnion freeze processes and dried</th>
<th>Amnion Freeze Processes-dried + LMWHA</th>
<th>Rates p</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>5</td>
<td>5.83±0.35a</td>
<td>5.89±0.66a</td>
<td>6.50±0.35b</td>
<td>0.051</td>
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<tr>
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<td>7.61±0.44a</td>
<td>10.50±0.51b</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
The increase in the number of epithelial layer was formed in the current amnion fresh on the day to-3 (5.00±0.60) and on the day to-5 (5.83±0.35) were not significantly different but different means to the day to-7 (8.33±1.46) with p<0.0001. In the current amnion freeze -dried epithelial cells lining the increase in the number of different meaning in every time the observation on the day to-3 (3.44±0.27), day 5 (5.89±0.66) and the last day to-7 (7.61±0.44) with p<0.0001. Same thing also happened in the current amnion freeze-dried + LMWHA where day 3 (4.89±0.62) is different means with day 5 (6.50±0.35) and is also different means to the day to-7 (10.50±0.51) with p<0.0001 (Pict. 1; see Table 2).

Table 3. The difference thick epithelial layer on observation day 3, 5 and 7 between groups treatment (unit: micrometer)

<table>
<thead>
<tr>
<th>Thick epithelial layer Day</th>
<th>Observation Time</th>
<th>Rates p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amnion fresh</td>
<td>3</td>
<td>0.52±0.1a</td>
</tr>
<tr>
<td>Amnion freeze-dried</td>
<td>5</td>
<td>0.53±0.14</td>
</tr>
<tr>
<td>Amnion freeze-dried + LMWHA</td>
<td>7</td>
<td>0.84±0.25a</td>
</tr>
</tbody>
</table>

Table 4. Differences thick epithelial layer in each group treatment between time watching the 3.5 and 7 micrometers (unit)

<table>
<thead>
<tr>
<th>Thick epithelial layer</th>
<th>Observation Time</th>
<th>Rates p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amnion fresh</td>
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<td>0.52±0.1a</td>
</tr>
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<td>Amnion freeze-dried</td>
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</tr>
<tr>
<td>Amnion freeze-dried + LMWHA</td>
<td>7</td>
<td>0.84±0.25a</td>
</tr>
</tbody>
</table>

On closer examination day 5 groups amnion freeze-dried + epithelial cells have a layer LMWHA thicker but not different means the 0.63±0.12 micrometer group that amnion fresh (0.53±0.14 micrometer) and the amnion freeze-dried (0.59±0.11 micrometer) with the p=0.385. While between groups amnion fresh and groups amnion freeze-dried is also not different means (p>0.05) so that the observation day 5 of the three groups not found differences thick layer cell that formed means.

On closer examination day-7 group amnion freeze-dried + LMWHA have the thickness epithelial cells layer (1.01±0.11 micrometer) who surpassed group fresh amnion (0.84±0.25 micrometer) and the amnion freeze-dried (0.61±0.03 micrometer). Statistical epithelial cells thickness between group amnion fresh and amnion freeze-dried + LMWHA not different means. But the thickness between these two groups were different means with the group amnion freeze-dried by a p=0.002.

The increase thick epithelial layer that is formed in the current amnion fresh on the 3rd day (0.52 ±0.1 micrometers) and on the 5th day (0.53±0.14 micrometer) were not significantly different but different means to the 7th day (0.59±0.11 micrometer) with p=0.008. In the current amnion freeze-dried epithelial cells lining the increase in the number of different means on observation on the 3rd day (0.36 ±0.1 micrometers) and the last 5th day (0.59±0.11 micrometer) with p<0.0001. While between the 5th and 7th day (0.63±0.12 micrometer) were not significantly different with p>0.05. In the current amnion freeze-dried epithelial cells + LMWHA thickness is formed between the time observation significantly different, where day 3 (0.37±0.09 micrometer) is different means with day 5 (0.63±0.12 micrometer) and is also different means to the day 7 (1.01±0.11 micrometer) with p<0.0001 (Pict.2; table 4).

Figure 2. Thick price epithelial layer is formed between groups treatment at various times observation
two other groups have not yet been found maturity. In the current amnion freeze-dried + LMWHA found maturity early more namely 83.3% compared to amnion freeze-dried (66.7 percent) and group fresh amnion (33.3 percent).

But these differences are statistically does not mean. On the day 5 maturity information found in 100% group amnion fresh, while in the current amnion freeze processes-dried and amnion freeze processes-dried + LMWHA smaller (66.7 percent and 83.3 percent) although differences were not significant. On the day 7 all the groups have already experienced maturity information (see Table 5).

DISCUSSION

Using fresh amnion as a wound cover that has high value more than just a biological closing wound will have been undoubtedly. The growth factor are contained in it is believed to have to give more epithelization facilitation and wound healing (Koizumi et al 2000, Wolbank et al 2009). In the next development using fresh amnion for clinical applications practical turns out to meet many obstacles. Starting with the availability of donor of fresh amnion that meets certain conditions to the recent research about the possible infection that can’t be avoided by because the nature of biological fresh amnion. Since that developing techniques and research to preserved fresh amnion so that, it is easy to be kept and can be used at any time with guarantee sterility that meet the standard. Questions arise when amnion was clinical applied in the state is not fresh or in a preservation. It is proven that treatment during preservation break epithelial cells lining membrane amnion and reduce the substrate containing important substrat and growth factor inside (Koizumi et al 2000, Effendi et al 2009, Cooper et al 2005, Wolbank et al 2009).

The research by Shay et al (2009) prove that there are high Hyaluronat macromolecule content (until 6000 kda) in fresh amnion leading to curiosity of researchers to examine further benefit combination topical Hyaluronat type low molecular weight (LMW) and amnion that preserved by freeze-dried as clinical process to epithelization superficial wound comparison with fresh and amnion freeze-dried on the individual white rat. The Election Hyaluronat LMW for combined with amnion freeze-dried in this research because it relates to the fact that literature study Hyaluronat LMW which is a degradation of Hyaluronat macromolecule which is proven to be in or invitro spur epithelization directly in epithelial cells skin (keratinosit) through the receptor CD44 and RHAMM, and not directly through increased production some growth factor by the macrophages are found in bed wound. On closer examination the first day you can see the establishment of epithelial have not yet been found in all the groups. This is in accordance with a number of studies (Papakonstantinou et al 1998, West & Fan 2002, Kennedy et al 2002) that initial effect of Hyaluronat LMW that promotes angiogenesis in dermal matrix itself began to be detected in the day 2 since giving and lasted until eight days later.

In the fresh amnion contains Hyaluronat macromolecule in the first phase that was hampered proliferation epithelial cells, endothelial and the macrophages are marked by the high levels of TIMP. This was sent down (moderate) inflamed acute phase where the PMN inflammation cells domination more visible than inflamed mononuclear cells (Shay et al 2009, Koh et al 2005). Contains a growth factor especially Epithelial Growth factor (EGF), Keratinocyte Growth factor (KGF), Hepatocyte Growth factor (HGF) that is contained in inert atmospheres, according to Koizumi et al (2001) is believed to spur epithelization directly without mediated by the macrophages although it has not yet seen in the first day.

On closer examination day 3 epithelial cells forming started can be observed in all the groups. In the current amnion fresh epithelial cells look total layer (5.00±0.60) and the thickness epithelial layer (0.52 ±0.1) superior means compared by the amnion freeze-dried (the amount of cell layers 3.44±0.27 thickness 0.36 ±0.1). In the current combination amnion freeze-dried and Hyaluronat LMW number of cells layer are no different from means (4.89±0.62 vs 5.00±0.60) with the group fresh amnion but the thickness layer superior in the fresh amnion (0.37±0.09 vs 0.52 ±0.1). This was Hyaluronat macromolecule that was found in fresh amnion in general is as space filling molecules in matrix interstitial dermis and the epithelial cells (Sakai et al 2000).

Table 5. Differences maturity epithelial layer between groups treatment at the time watching the 1,3,5 and 7

<table>
<thead>
<tr>
<th>Thick epithelial layer</th>
<th>Observation Time day to</th>
<th>Rates p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Amnion fresh</td>
<td>0.52±0.1a</td>
<td>0.53±0.14 a</td>
</tr>
<tr>
<td>Amnion freeze-dried</td>
<td>0.36±0.1a</td>
<td>0.59±0.11b</td>
</tr>
<tr>
<td>Amnion Freeze-dried + LMWHA</td>
<td>0.37±0.09a</td>
<td>0.63±0.12b</td>
</tr>
</tbody>
</table>
Hyaluronat have the ability to make a high water and act as shock-absorbers to trauma from the outside, beside the ability to improve hemidesmosome in interstitial spaces that began to appear on the day 3 (Chung et al 1998, Plopper 2007). This is causing the thickness epithelial layer in the current amnion fresh more evident. The increase levels Hyaluronat macromolecule this would reach the peak on the day 3 or 4 and is gradually decreased because the degradation process (Asari et al 1996, West & Fan 2002). Mitosis epithelial cells basalt that happened in the current amnion fresh higher was because there was a direct effect growth factor that contained inert in it. EGF and TGFβ proved to encourage proliferation and epithelial cell differentiation, increase epithelial layer thickness and its maturation (Pasonen-Seppänen et al 2003). In the current amnion freeze-dried influence growth factor against zmiotis is smaller because a decrease in the level growth factor of preservation. While in the current combination amnion freeze-dried + Hyaluronat LMW mitosis that occurred due to a combination of growth factor degree is decreased and direct effect of Hyaluronat LMW in CD44 receptors and RHAMM in epithelial cell and endothelial. Additional Hyaluronat LMW on the day 3 proved to be spur mitosis cells keratinosit beyond single application amnion freeze-dried and resembles epitelization in fresh amnion in spite of the different thickness cells lining that means.

On closer examination day 5 it appears that changes of thickness and total epithelial cells layer was formed in the current amnion fresh not significantly different with the cell layers (the number of 3: 5.83±0.35 vs 5.00±0.60; thick layer: 0.53±0.14 vs 0.52±0.01). Thus in the current combination amnion freeze-dried + Hyaluronat LMW took the increase of thickness and epithelial cells lining in significant portion compared with day 3 (the amount of cell layers: 6.50±0.35 vs 4.89±0.62; thick layer: 0.63±0.12 vs 0.37±0.09). Similarly, in the current amnion freeze-dried (the amount of cell layers: 5.89±0.66 vs 3.44±0.27; thick layer: 0.59±0.11 vs 0.36±0.1). A comparison of epithelial layer on the three groups showed that on the day 5 group combination amnion freeze-dried + Hyaluronat LMW superior significantly compared that other two groups (6.50±0.35 vs 5.89±0.66 vs 5.83±0.35) while the proportion thick epithelial layer in the current combination amnion freeze-dried +Hyaluronat LMW superior to the other of the group members but did not significantly (0.63±0.12 vs 0.59±0.11 vs 0.53±0.14). In the current fresh amnion it appears that the level Hyaluronat macromolecule that has reached the top do the inhibition in such proliferation basal keratinosit cells and macrophages through TIMP so an increase in the number of epithelial cells decreased and began to take place degradation Hyaluronat macromolecule to become a small molecules which (low molecular weight) that to be ready for activating epithelial cells, continued on endothelial and macrofag and fibroblast (West & Fan 2002, Jenkins et al 2005, Koh et al 2005). While in the current combination amnion freeze-dried + Hyaluronat LMW has happened activating since earlier where the degradation Hyaluronat macromolecule that is in a wound occurs more extensive caused by adding Hyaluronat LMW and there is a drop levels slows down TIMP (West & Fan 2002, Kennedy et al 2002).

On closer examination day 7 appears that the number of epithelial layer that formed increases significantly in all the groups that on the day 5. But from the group combination amnion freeze-dried + Hyaluronat LMW total layer formed cells more significantly than two other members of the group (10.50±0.51 vs 7.61±0.44 vs 8.33±1.46). The increase of thickness epithelial layer that is formed in the day 7 is also different significantly that in the day 5 except in the current amnion freeze-dried. The thickness of epithelial layer was formed in the current combination amnion freeze-dried not differ significantly compared to fresh amnion group (1.01±0.11 vs 0.84±0.25) but different significantly with the thickness epithelial layer in the current amnion freeze-dried (0.61±0.03). Overall, in the combination of amnion freeze-dried + Hyaluronat LMW epithelization process both in the increase in the number of epithelial layer and the thickness epithelial layer going as cuvillienar. While in the fresh amnion and amnion freeze-dried there is a plateau phase. In the fresh amnion happened between the 3th and 5th where it happens inhibition effect on the ceratinocyce cell, endothelial and macrophage of Hyaluronat macromolecule through TIMP that has reached the peak on the 3th and 4th and then degraded gradually transform into the LMW forms of inhibitory effects back into keratinocyte cell activation, macrophage and fibroblast accompanied drop levels of TIMP drastically. Activating LMW said specifically to improve the structure hemidesmosom and desmosom in intercellular matrix of keratinosit cells earlier (Chung et al 1998, Asari et al 1996). The structure hemidesmosom and desmosom in epithelial layer is proven to form integrity of epithelial cells structures which are betting each bind strong and keep epithelial layer is formed resistant to trauma (Plopper 2007). In the amnion freeze-dried plateau phase occurred in the addition of thick epithelial day 5th and 7th. This can be understood due to lack the Hyaluronat that is in amnion freeze-dried so that more limited ability structure formation of matrix intercellular which is rich desmosom and hemidesmosom and the ability to tie up water in the interstitial space.
Epithelial maturity that seems to be at the day 3 shows that the group fresh amnion where 50% superior has already reached maturity with more than one layer keratin is formed, while in the current amnion freeze-dried and the combination amnion freeze-dried + Hyaluronat LMW only formed early maturity and immature. Epithelial Maturity that go on the day 5 in the fresh amnion reached 100 percent, while can be obtained only 83.3 percent in the combination amnion freeze-dried + Hyaluronat LMW and 66.7 percent in the amnion freeze-dried. Maturity found 100 percent at all groups in the day 7. The continue of maturity that was marked by a keratin layer more than a layer shows that epithelial layer formed is more resistant to trauma (Gawkrodger 2003, Sterry et al 2006). Although the difference between groups are not significant, the tendency of accelerating epithelial cells differentiation and maturity in the fresh amnion was suspected to have been caused by working of growth factor especially EGF and TGF-β contained in a fresh amnion work directly with the ceratinocyte cells profile since the beginning because it is not found in the amnion freeze-dried in spite of additional Hyaluronat LMW.

CONCLUSION

Superficial wound care in white rats with the combination amnion freeze-dried and topical Hyaluronat low molecular weight give the epithelization speed that is superior compared to the treatment with single amnion freeze-dried in the day 3, 5 and 7. While it is compared to fresh amnion, epithelization speed little inferior on the day 3, but were leading in the 5 and 7.

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smooth muscle cells regulates their proliferation and migration. Glycobiology 8, 821-830