Review Article

Dentistry Section

Cryopreservation of Teeth: Freeze them Now to Use Later

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ABSTRACT

Cryopreservation refers to the storage of a living organism at an ultra-low-temperature in such a way that it can be revived and restored to the same living state as it was before it was stored. Recent studies have shown that mesenchymal stem cells (MSC) with the potential for cell-mediated therapies and tissue engineering applications can be isolated from extracted dental tissues. This article provides a complete review on the cryopreservation of teeth, its effects on the dental tissues and its use in dentistry. The application of this technology for the preservation of stem cells to be used for the regeneration of dental tissues has also been discussed.

Key Words: Cryopreservation, Cryoprotectants, Stem cells, Transplantation

INTRODUCTION

Clinical problems which are related to the loss and/or failure of tissues extend beyond dentistry to all fields of medicine. Currently, the replacement of lost or deficient tissues involves prosthetic materials, drug therapies, tissue and organ transplantation. However, all of these have limitations for example, the inability of synthetic prostheses to replace anything but the simplest structural functions of a tissue. Such problems have motivated the development of tissue engineering and stem cell therapy [1].

Personalized medicine is the most promising avenue for treating diseases that are known to occur throughout a lifetime. The loss of teeth may occur due to physical trauma, dental caries, periodontal disease or genetic defects. Missing teeth can cause several problems which are related to mastication, an aesthetic appearance, the lack of self confidence and the movement of other teeth into the existing space. Currently, there are many remedies which are available to replace the lost teeth such as prosthesis, implants, and the transplantation of teeth [2].

The transplantation of teeth depends on various factors such as extraoral time, stability of the cells, the surgical procedure, the handling of the tissues, the site of transplantation etc [3]. Sometimes it may not be possible to transplant the tooth immediately, when there is a damage at the recipient site in actively growing children etc. In such cases, the tooth has to be saved to be used later. The development of a technique which can allow individuals to use the tooth later, would be a highly desirable therapy.

The cryopreservation of teeth to be used for transplantation when needed is a promising and alternative choice for the replacement of the missing teeth. This article provides a complete review on the cryopreservation of teeth, its effects on the dental tissues and its use in dentistry. The application of this technology for the preservation of stem cells for use in the regeneration of the dental tissues has also been discussed.

CRYOPRESERVATION

Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, such as (typically) 77 K or –196°C (the boiling point of liquid nitrogen). At these low temperatures, any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped [4].

Bartlett and Reade were the first scientists to experiment on the cryopreservation of tooth material. Their experiments suggested that the cells in the teeth can survive the freezing process and that they can be cryopreserved [5].

THE PRINCIPLES OF CRYOPRESERVATION

To maintain long-term viability after a long-term storage, living cells must be brought into a state of suspended animation in which they can remain for indefinite periods of time and from which they can be brought back to viability at some point in the future. The temperature that is generally used for the storage of mammalian cells ie. 196°C, the temperature of liquid nitrogen appears to be adequate for these purposes.

At such a low temperature, water exists only in a solid state and no known biological reactions can take place [6]. Water solidifies into a crystalline structure which is known as ice when it is cooled below the freezing point. Because of the lesser density of ice as compared to water, ice crystals occupy a greater volume . As more water within the cells begins to solidify into ice, the shearing force and the pressure which is exerted on the intracellular organelles leads to considerable damage. Therefore, one of the principles of cryopreservation is the prevention of ice crystal formation [7].

As more and more water is converted to ice, the concentration of the unsolidified liquid phase increases. This can be toxic to the intracellular proteins and is known as ‘solution effects’. Avoiding this effect is the second principle of cryopreservation [7].

Rewarming of the cryopreserved tissue results in the melting of the ice and the release of free water, which reduces the osmolarity of the surrounding solution. Slow rewarming leads to thawing of the water and recrystallization causing further damage. On the other hand, rapid rewarming leads to a sudden drop in the extracellular osmotic pressure causing a rapid shift of free water in and out of the cell. This further leads to swelling and cell damage. This is

called 'osmotic shock', and its avoidance is a third major goal of successful cryopreservation [6].

In order to prevent these consequences from occurring, some chemical substances called as cryoprotectants are being used. The type, concentration, equilibration and the dilution of the cryoprotectant as well as its freezing rate, is known to influence the cryopreservation of the tissues. These components need to be optimized for specific cells and tissues.

The cryoprotectants can be classified into permeating and non permeating agents.

PERMEATING AGENTS[7]

These are small molecules which readily permeate the cell membranes. They impede ice crystallization by forming hydrogen bonds with the water molecules. At a high concentration they hinder the formation of ice crystals, leading to the formation of a solid glass like vitrified state where water solidifies but does not expand, thus preventing the formation of ice crystals. These agents also dilute the surrounding electrolytes, thus preventing the solution effect on the cells. Ex: propylene glycol (1,2 propanediol; PROH).

NON-PERMEATING AGENTS[7]

In contrast to the permeating cryoprotectants, nonpermeating cryoprotectants remain extracellular. They draw free water from within the cell and cause dehydration of the intracellular space. They reduce the formation of ice crystals when used along with permeating cryoprotectants and also play an important role during thawing. Therefore, the freezing and thawing protocols commonly use a high concentration of nonpermeating cryoprotectants during the thawing phase.

EX: SUCROSE

Cryopreservation of Teeth: Teeth which are extracted for orthodontic purposes, such as the impacted third molars and avulsed teeth have been used for the transplantation of the missing teeth [3]. However, sometimes conditions such as the severe damage of the recipient site, as in traumatic injuries or space loss, or the residual growth of the jaws in a developing child may pose problems during the transplantation. Hence, the cryopreservation of teeth in such conditions appears to be a promising tool.

Another important aspect which is related to the cryopreservation of teeth is the recovery of the stem cells that are known to be present in the dental tissues. The stem cells which are present in the pulp, and in the periodontal ligament are a potential source of undifferentiated mesenchymal cells, which can be used for the treatment of various diseases.

The process of cryopreservation is known to affect the survival of both the hard as well as the soft tissues.

THE EFFECT OF CRYOPRESERVATION ON THE PERIODONTAL LIGAMENT

The success of tooth transplantation depends upon the viability and the functionality of the cells of the periodontal ligament [8-14]. Various studies [15,16] have shown that the cryopreserved, transplanted teeth behave similar to the immediately transplanted teeth. The infiltration of the inflammatory cells along with granulation tissue formation was observed in the first week. The remaining cells of the periodontal ligament stained positive for alkaline phosphatase, thus suggesting the viability and the differential capacity of the remaining cells. Regeneration of the PDL was noticed in the second week. Ankylosis was not found and the alveolar bone and PDL formation was observed [16,17].

Although the reports suggested good periodontal healing, the regeneration process was slow as compared to that in immediately transplanted teeth. The temperature of the medium also is known to influence the healing of the PDL tissues. The damage to the PDL cells can be reduced by preserving them at –152°C than at 80°C [17]. The PDL also showed a normal histological structure after its preservation in liquid nitrogen for periods of 3 and 6 months [18].

The stem cells from the periodontal ligament could be retrieved from the cryopreserved cells. These cells maintained their characteristic features like the expression of surface markers and their potential for differentiation into adipocytes and osteogenic and cementum/PDL like tissue generation [18]. Temmerman et al, in his studies showed that the growth capacity, alkaline phosphatase activity and the membrane integrity of the PDL cells were similar in the cryopreserved and in the freshly extracted teeth [19].

ITS EFFECT ON THE PULP

The revascularization process in the immature and the mature teeth in the cryopreserved transplanted teeth was not impaired [20]. The quality of the vascular tissue which was formed was similar to the normal tissue in the pulp. However, the pulp did undergo necrosis, as it was found that the cryoprotectants were unable to diffuse into the pulp chamber. Hence, root canal treatment was considered to be mandatory in such teeth [20].

Other studies also suggested the differentiating capacity of the cells in the revascularised areas as they stained positive for alkaline phosphatase. The success of the revascularization was high in the incisors as compared to the premolars [16].

Various studies have reported the isolation and the retrieval of the dental pulp stem cells. The viability of the pulp stem cells was found to exist for 1 month to 2 yrs. The growth and differentiation and the expression of the surface markers in the stem cells from the cryopreserved teeth suggest that the cryopreservation procedure did not hamper the viability and functionality of the teeth.

THE CLINICAL APPLICATION OF CRYOPRESERVED TEETH

In clinical situations where immediate grafting is not possible, the teeth can be cryopreserved for future transplantation [21]. Traumatic injuries in children are increasing at an alarming rate. Avulsion is the most common traumatic injury which is observed in young growing children. Usually, teeth which are in the developing stages, where less than 1/3rd or 2/3rd of the root is formed are avulsed easily. Due to severe injury, the surrounding tissues may also be damaged and this may prevent the tooth from undergoing replantation . The cryopreservation of such immature teeth which can be used at a later date, may be a new promising treatment. This may also prevent the rejection of the graft.

Cryopreserved teeth can also be transplanted in growing children, as the differentiating capability of the PDL cells can induce the growth of the alveolar bone. The infraocclusion that can be observed when the implant is placed in a growing child can be prevented by transplanting cryopreserved teeth especially in adolescents [22,23].

The orthodontic movement of the cryopreserved teeth can be carried out, as these cells retain the capacity to regenerate PDL.

Stem cells can be isolated from the pulp and the periodontal ligament and can be used for the tissue engineering of the tooth or the treatment of other systemic diseases.

ITS APPLICATION IN PAEDIATRIC DENTISTRY

Stem cells from exfoliated deciduous teeth are known to be a potential source of various dental tissues. The exfoliated or extracted deciduous teeth can be cryopreserved and the stem cells can be isolated to be used for different purposes [24].

Like organ and stem cell banking, tooth banking is also evolving to produce a possible reserve of dental tissues. Avulsed incisors, impacted third molars, canines, premolars which are extracted for orthodontic purposes and exfoliated deciduous teeth can be banked.

THE RISKS WHICH ARE ASSOCIATED WITH CRYOPRESERVED TEETH

The problems of graft rejection and immunological reactions have not been addressed. The immune cells which are present in the pulp and the PDL can cause immunological reactions. The use of cryopreserved teeth in patients who suffer from systemic conditions also needs to be evaluated.

The risk of the transmission of blood borne diseases is also a topic of concern. The ethical issues which are related to the transplantation of cryopreserved teeth were also raised. The protocols and the procedure for the disinfection of the teeth before cryopreservation have not been identified and hence, the chance for infections after the transplantation cannot be ruled out. The medium which is used for storing teeth during the cooling also plays an important role. The medium itself may serve as an agent for an immune reaction and graft rejection [25].

CONCLUSION

New technologies have continually had a major impact on the dental practice, from the development of high-speed handpieces to modern restorative materials. Tissue engineering and stem cell therapy in the broadest sense, unquestionably, will affect the dental practice significantly within the next 25 years. As an interdisciplinary endeavour, these technologies will bring the power of modern biological, chemical and physical science to real clinical problems. At this time, science clearly indicates that the use of stem cells for the regeneration, reconstruction or the repair of the bone is feasible in principle. Substantial advances have been made in our ability to handle stem cells in the laboratory, and to exploit their inherent potential for building tissues. The translation of these advances into clinical practice will eventually occur. How rapidly this will happen, depends on solving the technical problems that are still significant. The kind of scaffold, the source of cells, the type of in vitro culturing, and the surgical procedure which is used—all require careful consideration.

The cryopreservation of cells and tissues is a promising tool for the preservation of cells which have to be used for such procedures. It is technique sensitive and many precautions need to be taken. This technology opens a new avenue for preserving one's own cells and tissues to be used at a later time.

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